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# EDITORIAL

On behalf of the organizing committees it is a great pleasure to welcome you in Paris for the 3rd International Symposium on Plant Signaling and Behavior.

The symposium, held from 29 June to 2 July 2015 on the campus of the University Paris Diderot-Paris 7, is under the auspices of the international society Plant Signaling and Behavior (PSB) and the French Society of Plant Biology (SFBV).

PSB 2015 takes place for the first time in Paris. This is an exciting opportunity for researchers around the world to gather and discuss the latest advances, the newest ideas and the potential for creative future research to understand the ways that plants sense, interact, communicate, learn, develop, survive and grow in an ever-changing world. We hope it will foster trans-disciplinary interactions among plant biologists, cell biologists, plant ecologists but also physicists and chemists and all researchers interested in the plant field

**François Bouteau**

On behalf of the organizing committees  
web site: <http://psb2015.com>

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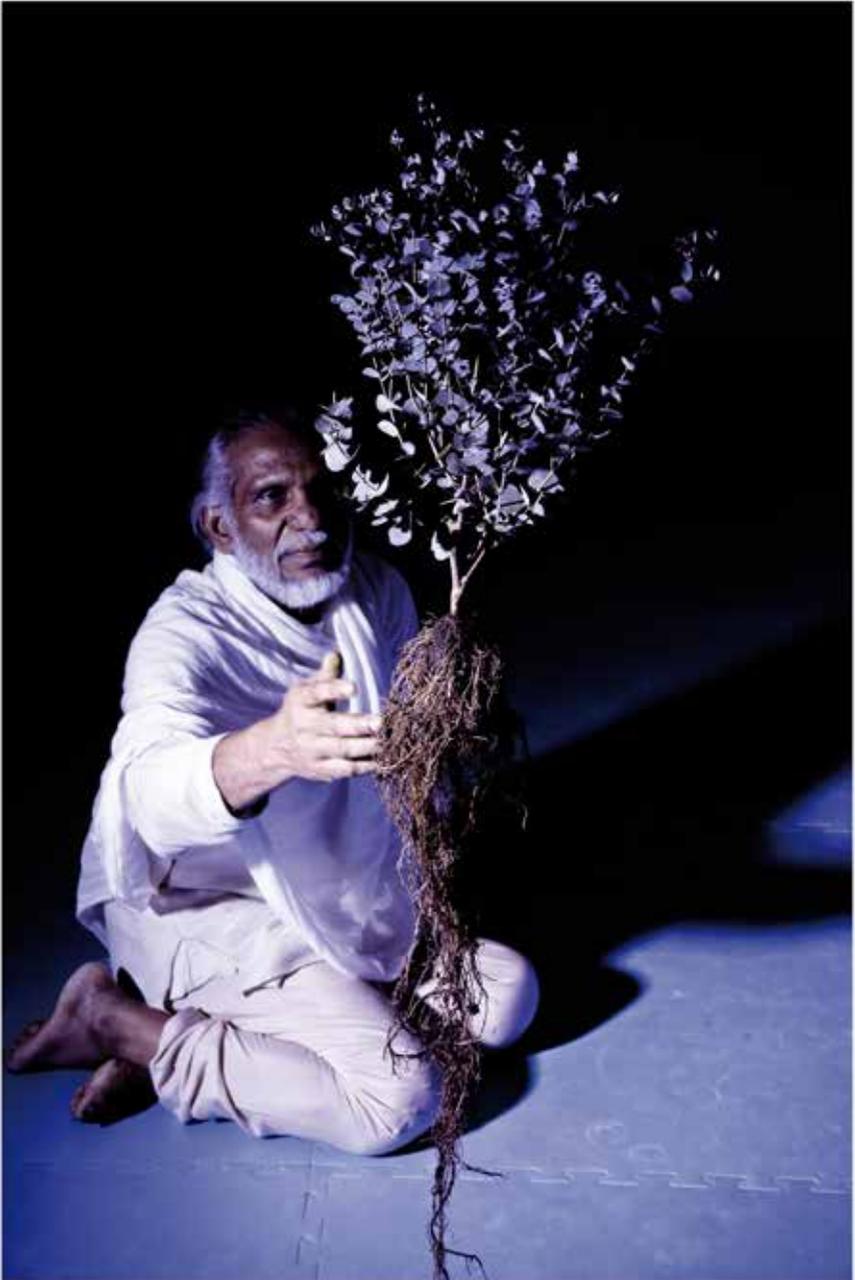
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# Oral presentation abstracts

# SESSION

# 1

## PLANT BEHAVIOR

### **Chairman Stefano Mancuso**

Understanding plant behavior and highlighting the structure of the information network that exists within plants is one of the main goals of the PSB society. This session will present some of the most recent advances in understanding how and what plants can perceive in their environment to adapt their behavior and discuss how such data can give rise to controversy over plant intelligence

## Abstract 1.1.

# CLAUDE BERNARD AND PLANT ANAESTHESIA

**Baluška F<sup>1</sup>, Yokawa K<sup>1</sup>, Mancuso S<sup>2</sup>**

Claude Bernard (1813-1878) is famous for introducing rigorous experimental methods to both medicine and biology. He was one of the first system biologist who considered organisms for integrated networks of hierarchical systems actively maintaining the constancy of their internal environment. But less known is that he followed steps of Alexander von Humboldt in his notion that plants, similarly as animals, are also living systems endowed with sensitivity to and activity towards their external environments. In order to support this unity of animals and plants, he was performing a number of experiments documenting sensitivity of plants towards the anaesthetics of his time, such as ether and chloroform. It is not well known, but the plant stress hormone ethylene is powerful anaesthetic. Stressed or wounded plants produce besides ethylene also ether. Moreover, our recent results show that plants are sensitive to both general and local anaesthetics. Even the chemically inert, monoatomic and generally unreactive noble gas xenon, which is excellent anaesthetic too, exerts anaesthetic actions on plants. Intriguingly, local root anaesthesia of Mimosa plants causes reversible loss of leaf responsivity to mechanical stimuli. This surprising finding suggest that the plant bodies are not only highly integrated but that root anaesthesia is switching off the active shoot behaviour. This finding is supporting the 'root-brain' hypothesis of Charles and Francis Darwin.

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## Abstract 1.2.

# AIRBORNE PLANT-PLANT SIGNALING LEADS TO A PRIMING EFFECT ON SALINITY TOLERANCE IN VICIA FABA L

**Caparrotta S, Boni S, Lanza M, Taiti C,  
Pandolfi C and Mancuso S**

Plants are exposed to many environmental stresses and adjust their physiological state in response to these factors. Being sessile organisms, plants evolved sophisticated mechanisms to transmit signals not only within but also between plants. For example, the defence mechanisms to biotic stresses, such as herbivores or pathogens, induce the release of volatile organic compounds (VOCs) which are able to elicit a response in neighbouring plants. Although the communication of biotic stress between plants is relatively well understood, very few studies deal with abiotic stresses. In order to observe the communication of abiotic stress among plants, we investigated whether VOC emission induced by salt stress in *Vicia faba* plants (the emitters) were able to alert neighbouring plants (the receivers) and elicit a stress response and/or an induction of the resistance to the stress itself. We applied a mild salinity stress to the emitters and we monitored the physiology of the plants for two weeks. The receivers were located in close proximity and shared the same environment with the stressed plants. At the same time unstressed plants, grown in a similar environment, were used as control.

We confirmed a significant reduction of photosynthetic rate and stomatal conductance as well as high accumulation of  $\text{Na}^+$  in the stressed-emitters. Concurrently, the relative growth rate (RGR) in the receivers was interestingly reduced compared to the control. After two weeks of treatment, also the receivers were stressed with a solution of NaCl, in order to investigate a possible effect of priming of the airborne signals on neighbouring plants. After stress RGRs of primed plants didn't change demonstrating that such plants were able to cope with the stress better than the controls.

Taken together our results suggest that VOCs produced by salt stressed plants trigger some form of priming mechanisms on the receivers, leading to an enhanced salt tolerance.

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## Abstract 1.3.

# ACOUSTIC SENSING OF BELOWGROUND PLANT GROWTH AND MONITORING OF FRUIT RIPENING

**Comparini D<sup>1,2</sup>, Iwase J<sup>3</sup>, Masi E<sup>4</sup>,  
Mancuso S<sup>2,4</sup>, Sato Y<sup>3</sup>, and Kawano T<sup>1,2,5</sup>**

Observation of roots, bulbs and tubers growing belowground is largely limited by the difficulty to monitor root growth and activity without removal of the soil. Dynamic growth and development of intact root systems or crops of interest are hardly monitored since plant organs growing in the soil cannot be detected or visualized by any conventional optical instruments. For this reason, non-invasive methods for detection of sub-terrestrial objects have been recently attracted the attentions by researchers who need a detailed insights into belowground properties. In our study, we attempted to apply acoustic means for detection of belowground plant growth by developing a novel non-invasive sensing technology based on propagation of frequency-modulated sound through the soil and its detection with acoustic band-pass filtering devices. This first attempt, whilst still in a developmental stage, successfully detected sweet potato tubers and undersoil tree roots. Lastly, this acoustic approach based on propagation and detection of sound between the distance with and without plant tissue was applied to study the ripening stages of tomato fruits. These primary attempts using acoustic techniques may induce further studies by scientists and engineers even in the field of ecological research, through developments of novel tools for belowground sensing and imaging.

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## **Abstract 1.4.**

# **GREEN THEN SMART - SPATIAL AWARENESS IN PLANT**

## **Mancuso S**

Somewhere between 400 and 1,000 million years ago, plants initiated a sessile lifestyle, taking advantage of the ubiquity of light as a source of energy as well as devising ways of compensating for body losses suffered because of browsing predators. Among the primary advances made by plants and sessile animals to survive predation was the evolution of different modular structures to ensure that in case of environmental damage or predation some module of the body may survive and regenerate the individual. In general, as a consequence of this primordial decision for a sessile and modular lifestyle, the specialization of tissues and cells in plants is minimised, if compared with animals, to limit predatory damages. Another consequence of the "sessile decision" was the need of a well-organized sensing system, which allows plants to explore efficiently the environment and to react rapidly to potential dangerous circumstances. Plants below and above ground are aware of the space surrounding them. Such responsiveness is indeed necessary to provide the appropriate actions in response to the many environmental stimuli. Example will be reported showing space perception in plants.

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## **Abstract 1.5.**

# **PLANT VOLATILES AS CODED SIGNALS IN DETECTION OF COMPETITIVE NEIGHBORS**

**Ninkovic V**

The most important factor affecting the growth of plant individuals is coexistence with neighboring plants. The research in this area has mainly been focused on competition between plants, but recently studies have shown that coexistence can take even other forms. In order to deal with proximate neighbors, rapid detection of diverse types of signals emitted by neighbors is essential for individual plants. Specific emission of volatile compounds as a natural consequence of plant physiological activity can be used as source of information by the individual plant in the detection of competitive neighbors. Plants have developed different strategies to exploit a range of phenotypic responses that enhance resource capture and avoid unfavorable competition. The induced responses may affect not only the plant, but also herbivores and their natural enemies that are associated with plants. It has been shown that volatiles released by damaged plants initiate defensive responses in neighboring plants making them less attractive for herbivores and more attractive for herbivore natural enemies. However, plant interactions by volatiles between undamaged plants have not previously been considered an important factor for induced resistance to herbivores.

Such chemical interactions have affects across three trophic levels; they affect plant biomass allocation, plant volatile emission, herbivore response and searching behavior of herbivore natural enemies. These results stimulate discussion on plant responses to their environment, not only in terms of 'chemical warfare' and allelopathy as a means for plant competition, but also as an advanced capacity of a plant to adapt to current conditions. It may be hypothesized that herbivores closely adapted to plant physiology are more sensitive to plant allelopathic responses, and that the third trophic level (predators of herbivores) respond to traits in the host plant.

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## **Abstract 1.6.**

# **THE INTELLIGENCE QUESTION**

## **Trewavas A**

In 1984, Barbra McClintock, Nobel prize winning plant biologist stated in her acceptance speech that " A goal for the future would be to determine the extent of knowledge the cell has of itself and how it uses that knowledge in a useful manner when challenged". The response to challenge is behaviour and thoughtful responses are intelligent responses. In 1937, Went and Thimann stated that "In tropistic movements, plants appear to exhibit a sort of intelligence; their movement is of subsequent advantage to them", thus relating intelligent behaviour in plants to fitness. The talk will discuss reasons that give rise to controversy over plant intelligence, provide suitable examples of this behaviour and will continue into areas like swarm intelligence that have organizational similarity to developing plants to see what useful information arises from viewing plants in this way.

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# SESSION

## 2

### **THE GREEN INDUSTRIES OF THE FUTURE, NATURAL, ARTIFICIAL AND HYBRID PHOTOSYNTHESIS**

**Chairman's Tomonori Kawano and  
Eric Herbert**

Photosynthesis is the central process allowing energy input in the biosphere through CO<sub>2</sub> fixation. The optimization of photosynthesis to enhance biomass or bioenergy production consists thus in an important research goal. This session will cover some of the most recent advances on natural, artificial and hybrid photosynthesis, and processes develop to improve biomass or bioenergy production.

## Abstract 2.1.

# INTRODUCTION TO NATURAL, ARTIFICIAL, AND HYBRID PHOTOSYNTHESIS RESEARCHES

**Kawano T**<sup>1,2,3</sup>

Recently, we have succeeded in developing a novel photosynthetic organism based on the mechanism mimicking the evolutionary processes through forced and accelerated symbiogenesis between non-photosynthetic protozoan cells and cyanobacterium. Furthermore, we have designed the artificial lighting conditions optimal for photosynthesis of living plants and algae. As above, photosynthetic organisms and its photosynthetic performance can be non-genetically engineered. In addition to natural photosynthetic models, our team has been engaged in development of artificial and hybrid photosynthetic model reactions which allows the reversible uptake and/or release of atmospheric carbon dioxide, followed by production and/or consumption of simple organic molecules in laboratory scale in vitro systems. One of our approaches towards such artificial photosynthesis research involves the development and use of organic and inorganic catalysts (artificial enzymes) designed after redox active natural enzymes or proteins. By aiming to develop a novel classes of artificial redox-active biocatalysts involved in production and/or removal of radical species (required for in vitro carbon assimilation), we attempted to understand and modify the natural catalytic proteins and functional nucleotide sequences of mammalian and plant origins. This presentation covers such attempts.

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## **Abstract 2.2.**

# **IS THERE HUMANITARIAN AND FINANCIAL CAPITAL IN PHOTOTROPIN SIGNALING?**

**Koskie K.A, Morrow J, Roberts Coats  
D, Leuchtman D, Holland J, Celaya  
R.B, Campbell T and Liscum M**

The phototropin blue light photoreceptors mediate a number of responses in plants that impact the optimization of photosynthesis and plant adaptation. A few years ago an interesting mutant allele of phototropin 1 (phot1), designated phot1- $\Delta$ PKD, was isolated that results in enhanced phot-dependent responses when present with a wild-type allele (in equal copy number). Arabidopsis plants stably heterozygous for both wild-type and  $\Delta$ PKD phot1 alleles exhibit a number of positively adaptive and 'productive' gain-of-function phenotypes when grown under greenhouse and field conditions. These phenotypes include, enhanced seedling survivorship, enhanced biomass production, accelerated flowering and increased seed production. Current efforts are aimed at determining the molecular mechanism(s) of these gain-of-function phenotypes, as well as translating this phenomenon into crop species.

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## Abstract 2.3.

# DISCUSSION ON THE MYSTERY OF BOYSEN JENSEN'S PHOTOSYNTHETIC RESPONSE CURVES UNDER PLANT CANOPY BY PROPOSING SIMPLE MATHEMATIC MODELS AND MINIMAL EXPERIMENTS

Okamoto Y, Kawano T

Photosynthetic irradiation (PI)-curves in most leafy plants are likely saturated under strong light when measured using a horizontally positioned single leaf. In 1932, Boysen Jensen has reported his observation that the PI-curve obtained with a horizontally placed single leaf of *Sinapis alba* L. showed saturation in PI-curve, and unexpectedly a stand of the same plant (a minimal canopy model) showed only linear increase response in PI-curve without attaining the saturation. This pioneering experiment induced a series of discussion if the nature of PI-curves in the plant canopy is largely differed from a single leaf and thus hardly attaining the level of saturation, indicating the importance of leaf angles and the degree of leaf overlapping in the plant stands or plant communities. However, the light source employed in the original work by Boysen Jensen (1932) was a position-fixed light bulb by which the light intensity could be readily altered by simply adjusting the distance between the light bulb and the leaves or standing plants. Here, we wish to point out some technical problems in the study of PI-curve in tall plants positioned under artificial light sources.

We assumed that the lack of saturation in PI-curves in a stand of *Sinapis* plant could be attributed to neither the movement of leaves nor the sun despite the suggestion by Monsi and Saeki (1953). Here, we propose a set of practitioner-friendly mathematical model (modified Hill-type equations) which could be applicable for estimating the total photosynthesis in the plant canopy structure consisted with inclined leaves, preliminarily testified with vegetables such as leafy lettuce. Our equations predict the photosynthetic capacity in the plant canopy structure simply through measurements of (1) the PI-curve, (2) the state of respiration, and (3) the light permeability (transmittance) in a single top-positioned leaf consisting the canopy structure.

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## **Abstract 2.4.**

# **SUSTAINABLE PHOTOSYNTHETIC SYSTEMS FOR BIOFUELS PRODUCTION**

**Patiño R**

In this work, a biorefinery based on photosynthetic cultures is proposed for biofuels production. Natural and agricultural wastes are one of the bases of the system. This biomass can be transformed to biogas by anaerobic fermentation, which later could be transformed to heat or mechanical energy. Electricity can be produced from local resources through photovoltaic cells or wind turbines. The intermittency of these systems could be solved using hydrogen as energetic vector. On the other side, solar outdoor cultures of microalgae are used for wastewater treatment. The microalgal biomass is used to produce some fine chemicals and to use the residuals as an additional source of bioenergy. Although the economical and ecological driving is mostly considered in the process, a social component is also proposed in order to reach the sustainability of the system. Therefore, educational and awareness campaigns should be considered to make the population conscious about the importance of the reduction in the everyday energy consumption, the use of local and renewable resources, and the principles of the biorefinery operating in the community. The hiring of local employers and their training on the processes of the biorefinery are also good practices to make own the project in the related population. Finally, despite the ecological and energy advantages of the proposed system, a high economical inversion is still required and only is possible if it is stated a compromise among the local inhabitants, the official authorities and the private initiative. In order to tackle this project, an interdisciplinary work should be performed with scientists from different

natural and social areas, engineers from a number of specializations, government stakeholders at different scales, and a group of entrepreneurs and investors.

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## Abstract 2.5.

# REPROGRAMMING PHOTOSYNTHESIS FOR OPTIMIZED BIOFUEL PRODUCTION IN MICROALGAE

## Peltier G

When facing adverse environmental conditions (such as nutrient limitation), microalgae adjust their physiological behaviour by stopping cell division and enhancing reserve accumulation. As a consequence, they convert the excess of reducing power generated by photosynthesis as storage compounds (starch or oil) or evacuate it as molecular hydrogen. These molecules of high energy content are of great biotechnological interest since they can be used as renewable feedstocks for green chemistry or biofuel production. However, the productivity of wild-type species needs to be improved in order to deploy economically viable biotechnological applications. Forward genetic approaches have been developed in our laboratory in the model microalga *Chlamydomonas reinhardtii* in order to discover new regulatory components controlling photosynthetic energy conversion and storage, and to identify new targets for biotechnological improvements. From the screen of an insertion library based on the analysis of chlorophyll fluorescence transients, a mutant (*pgrl1*) affected in cyclic electron flow around PSI was isolated. While growth of the *pgrl1* mutant is normal under most conditions, this mutant showed high hydrogen production abilities. By another way, a mutant (*std1*) impaired in a dual-specificity tyrosine-phosphorylation-regulated kinase (DYRKP1) was isolated from the screen of a mutant library based on starch accumulation kinetics. The *std1* mutant accumulates much more starch and oil than its wild-type progenitor in response to nutrient deprivation.

The DYRKP1 kinase is proposed to act as a negative regulator of reserve accumulation in response to the nutrient and energy status of algal cells (Schulz-Raffelt *et al.* submitted). Based on the study of a few algal mutants, we will discuss *i.* how photosynthetic energy conversion and storage are regulated to allow photosynthetic organisms to optimize survival under fluctuating environmental cues, and *ii.* how the engineering of regulatory components may help to domesticate microalgae by improving productivity under controlled growth conditions.

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## **Abstract 2.6.**

# **PHOTOBIOREACTORS DESIGN TO MAXIMIZE THE PRODUCTION OF MICROALGAE BIOMASS**

## **Sales EA**

In order to achieve economic viability and sustainability in the large-scale production of microalgae biomass, important physiological and technological drawbacks must be overcome. Currently, commercial production makes use of external open systems as an open basin, mainly due to the low cost of maintenance and construction. However, control of important parameters for culture is difficult, and productivity can be significantly lower. A suitable alternative for the control of key parameters to achieve higher productivity is the use of photobioreactors. The main challenges for biomass production in closed photobioreactors are not limited only to the cost. Also, the photosynthetic efficiency of microalgae depends on the balance between the exposure to light and temperature, among other parameters; and the system design must lead the culture medium within an optimum range of the light exposure and temperature where the biomass concentration can be maximized during the day. The research group LABEC has developed closed photobioreactors of various models, and some are presented in this work, as well as some studies on microalgae species subjected to nitrogen deficiency and light excess, in order to evaluate the production of carotenoids in each state, aiming to know the protein profile, to observe expression of proteins that may be related to each type of stress response.

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## Abstract 2.7.

# CHEMICAL ENGINEERING APPROACH FOR ARTIFICIAL PHOTOSYNTHESIS SYSTEM

**Uezu K<sup>1</sup>, Kawano T<sup>1</sup>, Comparini D<sup>1</sup> and  
Uchimura O<sup>2</sup>**

We will introduce the very interesting process that carbon dioxide in the air and water are directly reacted to produce hydrocarbons under mild conditions in the presence of several types of catalysts, and to show the process of verification from the chemical engineering point of view. About three years ago, Mr. Uchimura reported to us that water was converted to fuel hydrocarbons through the specific process (which was obviously not likely to happen, of course). We have carefully investigated about the process for one year and concluded that methanol dissolved in water is the carbon source producing hydrocarbons. However, later a similar phenomenon was observed even when the amount of methanol in water was very small. So, we re-verified according to the hypothesis that carbon dioxide in the air might be the carbon source of fuel hydrocarbon products. After the expected results were obtained in several experiments, we have been developing much higher efficient "artificial photosynthesis process" that carbon dioxide in the air is reacted with water to produce organic compounds.

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# SESSION

## 3

### AT THE INTERFACE OF PHYSICS AND PLANT BIOLOGY

#### Chairman Jean Marie Frachisse

For centuries, although they belongs to two distinct research communities, botanists and physicists, have joined hands and collaborate. As example, Leonardo da Vinci was inspired in fluid mechanics by observing the cross-sectional areas of tree trunks and his drawings illustrating the concept of a parachute and an auto-gyroscopic was based on plant observation. Three decades ago, with the genomic era, the vast majority of plant studies were devoted to the identification and dissection of gene regulatory pathways. It is only recently that physics back on stage and is progressively reintegrated into plant study. Therefore the role of physics is being increasingly revisited in plant biology taking advantage of new technical developments such as micromechanics and imaging technologies.

## Abstract 3.1.

# LONG DISTANCE SIGNALLING OF MECHANICAL STRESS IN TREES: EVIDENCE OF HYDRAULIC PULSES

**Badel E<sup>1,2</sup>, Guena G<sup>3</sup>, Louf J.F<sup>3</sup>, Tixier  
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Forterre Y<sup>3</sup>**

Plants are constantly subjected to external mechanical loads such as wind, rain or even neighbours. These stimuli are known to affect the growth of the plants, a process called thigmomorphogenesis. Typically, the bending of a tree stem leads to a local increase of the radial growth while the primary growth is rapidly stopped, suggesting a transport of the information from the stimulated zone to the apical zone. Among the different hypothesis found in the literature to account for this long-distance transport (electrical signal, hormone transport), it has been proposed that local mechanical stimuli, like bending, could generate an hydraulic signal that could move through the xylem.

Using stems segments, we displayed that bending strains generate a transient high pressure variation that is able to propagate rapidly along the hydraulic network. We quantified these pressure pulses and we observed i) that living cells were not involved in the phenomenon, ii) an inter-specific variability of its magnitude. Using a biomimetic approach and poroelastic artificial beams, we analyzed the physics of phenomena. We concluded that these hydraulic pulses are driven by the poroelastic properties of the saturated wood material and we proposed a physical modeling to explain the mechanism: the stem bending generates a variation of volume of the conduits, that expulses the incompressible water.

This generates a local transient hydraulic overpressure. *In planta* experiments confirmed that hydraulic pulses propagate along the vascular system of the xylem symmetrically to the upper and lower regions of the stem. Finally, molecular analysis in the apical part of trees showed the evidence of a remote signalling induced by the bending of the basis of their stem.

As a signaling process, this hydraulic behavior could be an efficient candidate for a fast long distance signal transporting mecanobiological information to the extreme organs as leaves, roots and apices.

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## Abstract 3.2.

# MECHANICS OF SINGLE PLANT CELLS

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Asnacios A<sup>1</sup>**

Cell mechanics is involved in many fundamental biological processes such as development and morphogenesis. Plant and animals have evolved different strategies for their development. Whether this is linked to major differences in their cell mechanics remains unclear, mainly because measurements on plant and animal cells relied on independent experiments and set-ups. We will present here recent measurements obtained with the same custom-made micro-rheometer to compare animal and plant cell rheology. This setup allowed us to determine the mechanical properties of single cells by performing dynamical oscillation tests. We found that wall-less plant cells exhibit the same weak power law rheology as animal cells, with comparable values of elastic and loss moduli. Remarkably, microtubules primarily contributed to the rheological behavior of wall-less plant cells whereas rheology of animal cells was mainly dependent on the actin network. Thus, plant and animal cells evolved different molecular strategies to reach a comparable cytoplasmic mechanical core, suggesting that evolutionary convergence could include the internal biophysical properties of cells. Besides measurements on protoplasts, we also characterized the mechanical behavior of single plant cells retaining their cell wall. The plant cell mechanical response was mainly elastic with a modulus of  $\sim 10^6$  Pa.

Decreasing the turgor pressure, the elastic modulus decreased accordingly before reaching a plateau value corresponding to the mechanical response of the cell-wall (plasmolysis). Comparing this behavior to that of a passive pressurized shell (soccer ball!), we reveal the way single plant cells adapt their internal pressure to changes in the osmolarity of their environment.

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### Abstract 3.3.

## HOW PLANTS FEEL GRAVITY: A FORCE OR POSITION SENSOR?

**Forterre Y<sup>1</sup>, Chauvet H<sup>1,2,3</sup>, Legué V<sup>2,3</sup>,  
Moulié B<sup>2,3</sup>, Pouliquen O<sup>1</sup>**

Gravity perception by plants plays a key role in their development and adaptation to environmental change, from the direction of seed germination to the control of the final posture. A crucial step in this gravisensing occurs in specific cells, the statocytes, which contain small grains of starch called the statoliths. The grains being denser than the surrounding intracellular fluid, they sediment, and give the direction of gravity. The key role played by the statoliths in the gravity perception is generally accepted as mutants with reduced starch content display reduced gravitropic response, whereas mutants with excess starch display an enhanced response. However, the cascade of mechanisms involved from the sedimentation of the statoliths to the change in direction of the organ is the subject of much debate. In particular, the precise gravi-response at the macroscopic scale, the exact nature of the stimulus and the link with the dynamics of the statoliths at the cellular level remains poorly understood. To address these questions, we have studied the macroscopic response of shoots to gravity using an original set-up based on a rotating table and a vital imaging of growth kinematics. Thanks to the centrifugal force, the response function of the plants (wheat coleoptiles, lentil shoots, *Arabidopsis* inflorescences, sunflowers) over a large range of inclination angle and gravity intensity ("g levels") is obtained and the gravisensitivity is estimated. Surprisingly, we found that the response of the plants to steady g levels is independent of the gravity intensity, depending only on the inclination of the plant.

This result suggests that the statoliths *position*, and not the *force* applied on the cell membrane, is the relevant internal signal that triggers the gravity response. In-situ visualization of the statoliths dynamics at the cellular level confirms that the statoliths behave as a "liquid" inclinometer, despite their granular nature.

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## Abstract 3.4.

# GREEN THERMOELECTRICS: OBSERVATION AND ANALYSIS OF PLANT THERMOELECTRIC RESPONSE

**Goupil C<sup>1</sup>, Ouerdane H<sup>1,2</sup>, Khamsing A<sup>1</sup>,  
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Patiño R<sup>1,6</sup>, Lecoeur P<sup>7</sup>**

Plants are sensitive to thermal and electrical effects; yet the coupling of both, known as thermoelectricity, and its quantitative measurement in vegetal systems never were reported. We recorded the thermoelectric response of bean sprouts under various thermal conditions and stress. The obtained experimental data unambiguously demonstrate that a temperature difference between the roots and the leaves of a bean sprout induces a thermoelectric voltage between these two points. Basing our analysis of the data on the force-flux formalism of linear response theory, we found that the strength of the vegetal equivalent to the thermoelectric coupling is one order of magnitude larger than that in the best thermoelectric materials. Experimental data also show the importance of the thermal stress variation rate in the plant's electrophysiological response. Therefore, thermoelectric effects are sufficiently important to partake in the complex and intertwined processes of energy and matter transport within plants.

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## **Abstract 3.5.**

# **CARNIVOROM – TOWARDS THE SENSES OF THE DARWIN PLANT**

**Hedrich R**

Predation plays a major role in energy and nutrient flow in the biological food chain. Carnivory is best known from the animal kingdom, but the plant kingdom has flesh eaters as well. The Venus Flytrap (*Dionaea muscipula*) is a carnivorous plant recognized as such by Darwin. Living on nutrient poor soils, *Dionaea* requires its flesh diet to counterbalance the malnutrition. Prey getting in contact with the touch-sensory organs protruding from the trap surface activate mechano-sensitive ion channels and induce action potentials eliciting trap closure in a fraction of a second entrapping the visiting prey. Since the time of Charles Darwin, scientists have struggled to understand the sensory biology of this carnivore. We could show that prey-catching with *Dionaea* combines plant-specific signaling pathways, involving touch hormone JA and stress hormone ABA that trigger ion channels, action potentials, and Ca<sup>2+</sup> signals. Upon recognition of the prey's nature by motion and chemical stimuli, glands on the inner surface of this "green stomach" start secreting a lytic cocktail. Following prey digestion, the same gland-based endocrine system absorbs the animal-derived nutrients.

We are currently investigating the molecular nature of receptors, channels, and signaling pathways behind the mechano-electric (trap) contraction-coupling and behind the senses of touch and taste. To understand the genomic basis of these remarkable features, we sequenced *Dionaea*'s 3 gigabase (homo-sapiens-like) genome. By projecting transcriptomic data from the non-excitabile petiole and different trap tissues onto the genome, we created a functional resource to explore the genetic landscape of the flytrap. Our data suggest that nature and roots of flesh eating with Darwin plant evolved from predator defense towards the acquisition of animal-derived nutrients.

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## **Abstract 3.6.**

# **REGULATION OF PLANT HYDRAULICS BY HORMONAL AND ENVIRONMENTAL SIGNALS**

**Maurel C, Prado K, Li G, Grondin A, Rodrigues O, Verdoucq L, Tournaire-Roux C, Lucas M, Boursiac Y, Santoni V, Luu D-T**

Aquaporins of the Plasma membrane Intrinsic Protein (PIP) subfamily mediate multiple controls of plant tissue hydraulics. Their contribution to whole root hydraulic architecture, in combination with endodermal barriers and xylem vessels, is addressed using a combination of reverse genetics and mathematical modelling approaches. A strong regulation of aquaporins in lateral root primordia, based on auxin-dependent inhibition, was recently uncovered. We show that by regulating the spatial and temporal distribution of root tissue water transport, this mechanism facilitates lateral primordium progression through overlaying cell layers, and thereby promotes lateral root emergence. In leaves, three PIP isoforms, including *AtPIP2;1*, contribute to water transport in inner tissues. Light-dependent changes in phosphorylation of *AtPIP2;1* and expression in transgenic plants of aquaporin phosphorylation mutants showed that C-terminal diphosphorylation of this single isoform is necessary for light-dependent regulation of leaf hydraulics. Based on functional assays in epidermal peels, *AtPIP2;1* was also found to play a role in stomatal closure, specifically in response to abscisic acid (ABA). Recent data indicate that *AtPIP2;1* acts on guard cell water permeability, with ABA-dependent phosphorylation of the aquaporin at a specific site. The protein kinase and signaling intermediates involved will be discussed.

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## Abstract 3.7.

# FEELING OSCILLATIONS WITH MSCS-LIKE MECHANOSENSITIVE CHANNEL

**Tran D<sup>1</sup>, Girault T<sup>1</sup>, Guichard M<sup>1</sup>,  
Leblanc N<sup>2</sup>, Moulia B<sup>2</sup>, DeLangre E<sup>4</sup>,  
Allain JM<sup>5</sup>, Frachisse JM<sup>1</sup>**

For plants, wind represents the major stimulation responsible for recurring mechanical load from the environment. Mechanical stimulation induces short-term cellular responses, such as calcium, ROS, kinase variations and activation of mechanoresponsive genes followed by long-term responses consisting in structural reinforcement. At whole-plant level the effect of repetitive mechanical stimulation produces plant shortening, increasing of stem and trunk diameter. However at cellular level, perception and transduction of oscillatory mechanical stimulation is still elusive. We have shown that Mechanosensitive channel Small conductance-Like 10 (MSL10) contributes to oscillatory perception in plant. This channel responds to pulse membrane stretching with rapid activation and deactivation. Furthermore the activity of the channel is modulated by sinusoidal pressure stimulation with a higher open probability upon oscillatory than during sustained stimulation. At plant level, performing oscillatory mechanical stimulation of *Arabidopsis* stem, we evidenced the responsiveness of mechanoresponsive genes (ZAT7, ZAT12 and TCH4). Using knock-out mutant and overexpressing lines, we identify MSL10 to be necessary for ZAT7 gene activation by stem oscillation. Our results demonstrate that MSL 10 behave as a frequency transmitter. Therefore MSL10 is, to our knowledge, the first example of mechanosensor *per se* involved in early frequency perception in plant so far.

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# SESSION

## 4

### LONG DISTANCE SIGNALING

#### Chairman Patrice Thuleau

Plants, as sessile organisms, use multiple and sophisticated mechanisms to respond to their environment. In particular, in order to survive, they must perceive all the stresses they face, such as water or nutrient deficiency, pathogen attack, wounding, but more importantly they have to spread the information throughout the plant. Such systemic signaling involves a plethora of chemical regulators including hormones, peptides, RNAs, metabolites or ions and also electrical long-distance signals, all being coupled to sophisticated sensory systems. This session "Long distance communication in plants" presents some of the recent advances in this exciting area of plant sciences, describing some examples of the involvement of chemical and/or electrical signals and new sensory systems.

## **Abstract 4.1.**

# **A POSSIBLE CROSSTALK OF SEROTONIN AND MELATONIN WITH GLUTATHIONE REDUCTASE AND CATALASE ACTIVITY ACCOMPANYING LONG DISTANCE SENSING OF SALT STRESS IN SUNFLOWER SEEDLINGS**

**Kaur H and Bhatla SC**

Melatonin and serotonin are important endogenous free radical scavengers. They help in improving the redox state of cells by scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS). Melatonin can also upregulate the transcript levels, stimulate the activities of several antioxidant enzymes and regenerate endogenous antioxidants, such as glutathione. They may also directly scavenge  $H_2O_2$  and help in the maintenance of intracellular  $H_2O_2$  concentration at steady state levels. A reduction in the overall growth of sunflower seedlings in response to NaCl stress is significantly normalised by raising the seedlings in presence of 15  $\mu M$  of melatonin/serotonin (present work). This improvement coincides with two fold increase in the reduced glutathione (GSH) content in seedlings raised in presence of 15  $\mu M$  of melatonin relative to control (2 d old seedlings). Serotonin application also evokes similar response as melatonin, with minor quantitative differences. Although GSH content also gets elevated by melatonin/serotonin treatments in seedlings not subjected to NaCl stress and those subjected to serotonin treatment (irrespective of NaCl stress), the increase in GSH content is highest due to melatonin treatment. Melatonin treatment enhances glutathione reductase (GR) activity in the cotyledons from salt stressed seedlings. The enzyme activity

is, however, reduced in control seedlings. These observations indicate modulation of response due to melatonin/serotonin treatments by NaCl stress in elevating GSH content and altering GR activity in the seedling cotyledons. Although melatonin treatment leads to  $H_2O_2$  accumulation in 2 d old seedling cotyledons, it does not correlate with any increase in catalase (CAT) activity. Melatonin treatment, in fact, lowers catalase activity in cotyledons. Present findings put forward new observations on the differential response of glutathione reductase (GR) and catalase (CAT) to melatonin/serotonin treatments, thereby adding interesting information about their ROS scavenging roles in seedlings subjected to salt stress.

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## **Abstract 4.2.**

# **PROPRIOCEPTION AS A KEY CONTROL OF GRAVITROPIC MOUVEMENTS AND PLANT POSTURE IN THE AERIAL ORGANS OF PLANTS**

**Mouliat B<sup>1,2</sup>, Coutand C<sup>1,2</sup>, Leblanc-Fournier N<sup>2,1</sup>, Douady S.<sup>5,4</sup>, and Bastien R<sup>1,3</sup>**

Gravitropism, the turning movement of plants aligning to the direction of gravity is a salient trait of land plants. If its motors and early signaling have been thoroughly investigated, the control of the overall movement and its capacity to effectively align to the gravity direction has only started to be elucidated recently. This has been achieved by combining i) quantitative records of the kinematics of tropism in 11 plant species sampling angiosperms phylogeny and ii) a heuristic modelling of the dynamics and diversity of the bending and straightening process. It was shown that the sensing of the local inclination angle by statocytes is not sufficient to bring the organ back to the gravity direction after tilting. Alignment to the direction of gravity and proper control of the plant posture can only be achieved through a balanced cross-talk between gravisensing and a proprioceptive control, tending to straighten the organ. This balance is captured by a single dimensionless number B that fully controls the diversity of the movement and of the final posture of the plant. Moreover, our results suggest that the proprioceptive sensitivity has been selectively tuned to avoid the two pitfalls of tropism through differential growth: curvature fixing where growth stops and passive orientation drift.

A parsimonious universal model of the graviproprioceptive control of plant gravitropism, the AC model, has been developed, and found valid over the all species sample and two orders of magnitude of organ size. Further investigation has revealed that the proprioception of bending deformations during the tropic movement is consistent with the Sum of Strain-Sensing model (S3m) developed for strain-mechanosensing during bending by external loads. Finally a model-assisted, non-contact phenotyping of the gravi-proprioceptive balance B has been developed and used to screen mutants for graviproprioceptive control so to investigate the molecular genetics of its mechanobiological regulation.

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## Abstract 4.3.

# ELECTRICAL AND JASMONATE SIGNALING REGULATE DIGESTIVE ENZYME ACTIVITY AND PHOTOSYNTHESIS IN THE CARNIVOROUS PLANTS

**Pavlovič A**

Carnivorous plants grow in nutrient-poor habitat and obtain substantial amount of nutrients from prey capture and digestion. For this reason, these plants have evolved well developed sensory system for detection of prey in the modified leaves called traps. Recent data revealed that this sensory system was adopted from plant-defense mechanisms. Mechanical stimuli from prey trigger action potentials and are followed by accumulation of different jasmonate molecules in the trap: *cis*-12-oxophytodienoic acid (*cis*-OPDA), jasmonic acid (JA) and jasmonic acid isoleucine conjugate (JA-Ile). The mechanical stimuli alone are not sufficient for induction of high enzyme activity in digestive fluid. To trigger the full enzyme activity, chemical stimuli must be involved. The enzyme activity in the trap correlates with the tissue jasmonate concentration but not with other plant hormones. This double sensory system helps to optimize production of digestive enzymes, because only mechanical and chemical cues are indicator of live prey in the trap. External application of JA bypassed the mechanical and chemical stimulation and was able to induce full enzyme activity without any other stimuli. On the other hand, external application of other plant phytohormones well-known from plant-defense mechanisms, salicylic (SA) or abscisic acid (ABA), failed in this response.

This talk documents the central role of jasmonates in regulation of digestive enzyme activity in the carnivorous plant of the genus *Drosera* and *Dionaea* in response to different stimuli from prey. Moreover, the electrical and jasmonate signaling has significant impact on photosynthetic reaction in the traps of both genera.

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## **Abstract 4.4.**

# **NUTRIENT AND SALT STRESS SENSING BY ROOTS INDUCES LONG DISTANCE CALCIUM SIGNALLING IN LEAVES**

**Poitout A, Lanciano S, Sanchez F,  
Xiong TC**

Calcium ( $\text{Ca}^{2+}$ ) is well established as a second messenger involved in many signalling pathways. Biotic and abiotic stimuli induce  $\text{Ca}^{2+}$  signals within plant cells, which, when decoded, enable these cells to adapt in response to environmental stresses. Perception of stimulus is often sensed by some cells or tissue and propagation of information between cells/tissues necessary for plant systemic responses is crucial to face in time to the changing environmental conditions. Therefore the propagation of signals over long distances, for instance from roots to leaves or leaf to another leaf, could relay necessary information and might be vital for plant adaptations. The role of  $\text{Ca}^{2+}$  in long distance signalling is still unclear in plant. Using a BRET-based  $\text{Ca}^{2+}$  sensor, propagation of  $\text{Ca}^{2+}$  signals on leaves were imaged after stresses applied on roots. The results obtained with this  $\text{Ca}^{2+}$  reporter show that sensing of nutrients or salt stress triggers  $\text{Ca}^{2+}$  waves on leaves. Analysis of the  $\text{Ca}^{2+}$  signals of entire plant leaves show that only mature leaves response by  $\text{Ca}^{2+}$  signals. Characterization of these  $\text{Ca}^{2+}$  signals show different types of  $\text{Ca}^{2+}$  waves were generated and could be defined by their directions, velocities and localizations. The type of  $\text{Ca}^{2+}$  waves are stimulus-dependent suggesting that features of  $\text{Ca}^{2+}$  waves at tissue level are new components of  $\text{Ca}^{2+}$  signature that contribute to plant specific responses.

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## Abstract 4.5.

# LONG-DISTANCE ELECTRICAL SIGNALING IN BRYOPHYTA

**Trebacz K**

Bryophyta belong to plants which colonized lands as first. The hypothesis was verified that these plants adapted a signaling system based on ion fluxes, well developed in algae, to terrestrial conditions. Two liverworts: *Conocephalum conicum*, *Marchantia polymorpha* and the moss *Physcomitrella patens* have been chosen to perform a comparative study of long-distance signals - action potentials (APs). All these plants generate APs in response to different environmental stimuli. Responses to light and cold have been examined in detail. Cells of *C. conicum* generate typical all-or-none APs after illumination. Darkening evokes transient hyperpolarization. *M. polymorpha* and *P. patens* fire APs upon illumination although they are not as responsive as *C. conicum*. In addition, they generate long-lasting (>5 min.) action-potential-like responses after darkening, which consist of relatively fast depolarization followed by long-lasting plateau and slow repolarization. Membrane potential changes evoked by light are blocked by DCMU - an inhibitor of the photosynthetic electron transfer chain. Action potential-like responses depend on the activity of the electrogenic proton pump. They occur frequently under low energy conditions: in anoxia or after application of H<sup>+</sup>-ATPase inhibitors (FCCP, DCCD) and are eliminated after intensive illumination, aeration, or fusicoccin. Low temperature causes depolarization in all these plants. When the rate of temperature drop is high enough, APs are generated. They do not differ in shape and amplitude from those evoked by illumination, although their duration is significantly higher.

The ion mechanism of APs in Bryophyta plants, deduced on the basis of ion channel inhibitor experiments, consists of calcium and anion fluxes in the depolarization phase followed by potassium efflux during the repolarization phase.

The activity of ion channels participating in AP seem to depend on the energy status of cells since the threshold of excitation is lower in energy-starved plants. The same feature is observed in higher plants.

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## **Abstract 4.6.**

# **PLANT ELECTRICAL SIGNALING AND BEHAVIOR**

## **Volkov AG**

Electrical signaling on long and short distances exists in plants. There are five major types of electrical signaling in plants and animals: action potentials, electrotonic potentials, graded potentials, receptor potentials and streaming potentials. The action potential in plants can propagate over the entire length of the cell membrane and along the conductive bundles of tissue with constant amplitude, duration, and speed. Electrotonic potentials exponentially decrease with distance. A graded potential is an electrical signal that corresponds to the size of the stimulus. Receptor potentials are generated by mechanosensory ion channels. A streaming potential is a potential difference that arises across a capillary tube or membrane when a liquid is forced through it. Electrical signaling not only carries information from one part of the plant to another, but it also plays an important role in plant memory and circadian rhythms. Bioelectrical impulses can travel from the root to the stem, leaves and vice versa. Chemical treatment, intensity of the irritation, mechanical wounding, previous excitations, temperature, and other irritants influence the speed of electrical signal propagation. Electrostimulation of plants induces electrotonic potentials propagating along their leaves and stems. The instantaneous increase or decrease in voltage of stimulating potential generates a nonlinear response in plant tissue. Any electrostimulation that is not instantaneous, such as sinusoidal or triangular functions, results in linear responses in the form of small graded potentials. The amplitude and sign of electrotonic potentials depend on the polarity and the amplitude of the applied voltage during electrostimulation.

Using the synchronous electrostimulation of a leaf from different points, we studied the interaction between the electrotonic potentials. The information gained from this study can be used to elucidate the intracellular and intercellular communications in the form of electrical signals within plants. Plant behavior is a complex of responses to external or internal stimuli.

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# SESSION

## 5

### **PLANTS IN EXTREME ENVIRONMENT**

#### **Chairman Chedly Abdelly**

Plants have to face extreme environments from drastic limitation to excess of water leading to waterlogging. Extremophiles plants acquired through a strong natural selection pressure the required features to tolerate environmental constraints. At the fundamental level and considering the high genetic diversity available within these species, they constitute promising models for the identification of relevant traits involved in the tolerance to abiotic stresses. Some species have also economic interests (production of biomass that can be valued for human food, animal feed, biofuels, biomolecule production for pharmaceutical and food industries,...) and ecological interests (stabilization of saline soils, phytoremediation,...). Thus, characterization of these plants on the basis of these criteria would identify the most promising species for the transformation of marginal lands into productive systems.

## Abstract 5.1.

# HOW DOES THE HALOPHYTE *CAKILE MARITIMA* CONVERT SALT STRESS TO A BENEFICIAL SIGNAL?

**Abdelly C**

Tolerance of plants to abiotic constraints is greatly related to the rapid perception of stressors and to the ability to decode environmental signals. We investigate connections between osmotic, oxidative and hormonal signals in salt tolerant and salt sensitive species during early salt stress response. The behavior of a local halophytic species *C. maritima* to short-term severe salinity, in comparison with *T. salsuginea* and *Arabidopsis thaliana*, was studied. Results showed that salt signal perception depends on species and organs and varies depending on salt ion distribution. *C. maritima* as Na<sup>+</sup>-includer, predominately accumulated, proline and sugars in leaves associated with increased cytokinins levels, especially *t*-zeatin. This was strongly correlated with the osmotic adjustment. Activated auxin and gibberelin signals in both leaves and roots of *C. maritima* promote its growth under high salinity. Results showed also differential oxidative signal perception between halophytes and glycophytes. Salt stress is sensed in *A. thaliana* by a continuous imbalance of redox status and an extended repression of antioxidant systems. However, response of halophytes was characterized by an early and transient oxidative burst.

*C. maritima* was more able to perceive salt stress and to rapidly transmit this signal between organs than *T. salsuginea*. *C. maritima* displayed transient increase of H<sub>2</sub>O<sub>2</sub> and MDA which is followed by an activation of antioxidant defense which kept at high level in leaves and roots. H<sub>2</sub>O<sub>2</sub> signal stimulated ABA, JA and ACC accumulation, resulting in enhanced expression of ethylen response factor1 (*ERF1*), the convergence node of the JA and ethylene signaling pathway in both leaves and roots of *C. maritima* as compared to *T. salsuginea* and *A. thaliana*.

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## Abstract 5.2.

# LINKING OXYGEN AVAILABILITY WITH MEMBRANE POTENTIAL MAINTENANCE AND K<sup>+</sup> RETENTION OF BARLEY ROOTS: IMPLICATIONS FOR WATERLOGGING STRESS TOLERANCE

**Zeng F.R.<sup>1,2</sup>, Konnerup D<sup>3</sup>, Shabala L<sup>1</sup>,  
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and Shabala S<sup>1</sup>**

Oxygen deprivation is a key determinant of root growth and functioning under waterlogging. Changes in net K<sup>+</sup> flux and membrane potential (MP) of root cells were measured from elongation and mature zones of two barley varieties under hypoxia and anoxia conditions in the medium, and as influenced by ability to transport O<sub>2</sub> from the shoot. Oxygen deprivation results in an immediate K<sup>+</sup> loss from roots, in a tissue- and time-specific manner, affecting root K<sup>+</sup> homeostasis. Both anoxia and hypoxia induced transient membrane depolarization; the extent of this depolarization varied depending on severity of O<sub>2</sub> stress and was less pronounced in a waterlogging-tolerant variety. Intact roots of barley were capable of maintaining H<sup>+</sup>-pumping activity under hypoxic conditions while disrupting O<sub>2</sub> transport from shoot to root resulted in more pronounced membrane depolarization under O<sub>2</sub>-limited conditions and in anoxia a rapid loss of the cell viability. It is concluded that the ability of root cells to maintain MP and cytosolic K<sup>+</sup> homeostasis is central to plant performance under waterlogging, and efficient O<sub>2</sub> transport from the shoot may enable operation of the plasma membrane H<sup>+</sup>-ATPase in roots even under conditions of severe O<sub>2</sub> limitation in the soil solution.

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# SESSION

## 6

### **SIGNALING IN STRESS PHYSIOLOGY**

#### **Chairman Bertrand Gakière**

Due to their sessile nature, plants are constantly subjected to various abiotic stresses or pathogen diseases. The main goal of this session is to present and evaluate some of the most recent advances in understanding plant stress signaling, by bringing pioneering researchers in the field. A panel of talks will be presented, dealing with (i) toxicity of aluminum-rich environments, (ii) nutritional impact on resistance to pathogens, (iii) the use of elicitors to stimulate responses to stresses, (iv) the cross-talk between different signaling pathways occurring in response to salt stress and the nature of the exchanged signals.

## Abstract 6.1.

# **B-AMINO BUTYRIC ACID (BABA)-INDUCED RESISTANCE IN *ARABIDOPSIS THALIANA*: LINKS WITH IRON HOMEOSTASIS**

**Besson-Bard A, Koen E, Trapet P,  
Brulé D, Kulik A, Klinguer A, Atauri-  
Miranda L, Meunier-Prest R, Boni G,  
Glauser G, Mauch-Mani B,  
and Wendehenne D**

$\beta$ -aminobutyric acid (BABA) is a non-proteogenic amino acid potentiating plant defense reactions in many different species against a variety of (a)biotic stresses. Nevertheless, processes inherent to the effects of BABA remain poorly understood. We provided new elements that could contribute to the understanding of the mode of action of BABA. Based on its structure, we hypothesized and confirmed that BABA exhibits iron (Fe) chelating properties by forming a stable hexacoordinated complex *in vitro*. The Fe chelating properties of BABA led us to examine its impact on *in planta* Fe homeostasis. Intriguingly, the phenotype of *Arabidopsis thaliana* BABA-treated plants was similar to those of plants grown in Fe deficient conditions. We showed that BABA is a modulator of Fe homeostasis and leads to a transient Fe deficiency response in *A. thaliana* plants exemplified by the modulation of the expression of genes or proteins involved in Fe sequestration and transport including *AtNAS4*, one of the four *A. thaliana* genes encoding nicotianamine synthase (NAS) previously found to be up-regulated through a nitric oxide-dependent process. It seems that BABA impacts the availability or the distribution of Fe rather than its assimilation since this response was not correlated to decrease in Fe concentrations.

A metabolomic analysis indicated that BABA and Fe deficiency induced the accumulation of common metabolites including *p*-coumaroylagmatine, a metabolite previously shown to be synthesized in several plant species facing pathogen attack. Finally, we showed that the protective effect induced by BABA against the necrotrophic fungus *Botrytis cinerea* was mimicked by Fe deficiency.

These data support the occurrence of cross-regulatory interactions between iron homeostasis and immune responses.

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## **Abstract 6.2.**

# **NITRIC OXIDE CROSSTALK WITH REACTIVE OXYGEN SPECIES SCAVENGING ENZYMES IN MODULATING SALT STRESS IN SUNFLOWER SEEDLINGS**

**Bhatla SC**

High reactivity of nitric oxide (NO) radical induces different post-translational modifications in a wide range of proteins thereby affecting their structure and function. NO can interact with different metal centres in proteins such as heme-iron, zinc-sulphur clusters, iron-sulphur clusters and copper, resulting in the formation of a stable metal-nitrosyl complex or production of biochemical signals which ultimately lead to modification of protein structure/function. Generation of reactive oxygen species (ROS) is one of the major long distance stress markers evident in the cotyledons of sunflower seedlings in response to salt stress (120 mM NaCl) to which roots are exposed. Present investigations report differential modulation of various ROS scavenging enzymes by NO as an early signaling event in the cotyledons of 2 day old seedlings. Various ROS scavenging enzymes analysed can be grouped into three categories with reference to their modulation by nitric oxide (NO). Category I enzymes (superoxide dismutase and glutathione peroxidase) are unaffected by NO availability and the response is also not sensitized by salt stress. Category II enzymes (catalase and glutathione reductase) are inhibited by sodium nitroprusside as NO source and the response is sensitive to NaCl stress as well.

Category III enzymes (peroxiredoxin, peroxidase and heme-oxygenase) exhibit significant enhancement of activity in response to NO and the response is also sensitive to salt stress. Treatment with peroxynitrite is inhibitory for all the enzymes except peroxiredoxin in control (- NaCl) conditions.

The available data, thus, demonstrate sensing of salt stress through modulation of the activities of various ROS scavenging enzymes and their crosstalk with nitric oxide. Work is in progress to investigate the nature of NO binding and the possibility of reversibility of its impact on various ROS scavenging enzymes.

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## **Abstract 6.3.**

# **PLANT PERFORMANCE UNDER ABIOTIC STRESS CONDITIONS – PRIMING AGENTS TO THE RESCUE**

## **Fotopoulos V**

Abiotic stress factors represent key elements limiting agricultural productivity worldwide. Close examination of plant-to-plant communication in nature has revealed the development of unique strategies from plants for responding to abiotic stress, with one of the most interesting being through priming for improved defense responses. The process of priming involves prior exposure to a biotic or abiotic stress factor making a plant more resistant to future exposure. Priming can also be achieved by applying natural or synthetic compounds which act as signaling transducers, 'activating' the plant's defense system. Although the phenomenon has been known for many years, it has only been recently suggested that priming can enhance the tolerance of crops to environmental stresses in the field. Here an update will be provided on the research carried out at the Cyprus University of Technology using priming agents towards induced acclimation of plants to environmental challenges, focusing on the use of synthetic compounds such as NOSH-aspirin as potent regulators of plant stress responses.

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## **Abstract 6.4.**

# **AL STRESS SIGNALLING IN PLANTS: MODEL TO CROPS**

**Panda SK**

Aluminium (Al) is the third most abundant metal in the earth and becomes toxic in the soil only when the pH becomes acidic, i.e., 4.5-5.5. Al stress affects crop productivity in acidic soil upto 50 % and more than 40 % of world arable soil is acidic. Al affects root growth by inhibiting it and the distal transition zone in the root tip was found to be the major target of Al. Al induces reactive oxygen species (ROS) production in cell and other organelles and affects the mitochondrial function of root cells by depolarizing the inner mitochondrial membrane, ROS and  $\text{Ca}^{2+}$  burst that may ultimately result in cell death. Many key genes and transcription factors like ALMT1, MATE, ALS, STOP1, ART1, AOX1 etc. have been identified that participate in the tolerance mechanism of various plants like Arabidopsis, tobacco and crops, cowpea, blackgram and rice under Al stress. Understanding the Al stress signalling mechanisms in the model and crop plants can help in developing Al tolerant crops in future.

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## Abstract 6.5.

# ROLE OF NITRIC OXIDE IN GUARD CELLS DURING STOMATAL CLOSURE BY PLANT HORMONES AND ELICITORS

**Raghavendra AS**

Stomatal closure is an effective way of not only minimizing the loss of water into leaves but also restricting the entry of plant pathogens. In view of their dynamic responses, the stomatal guard cells are model systems to study the signaling cascade mechanisms in plants. Stomatal closure initiated by signals of either abiotic or biotic factors, is the result of the loss in guard cell turgor due to the efflux of  $K^+$  and anions. Exposure to abscisic acid (ABA) or elicitors initiates the rise in reactive oxygen species (ROS)/nitric oxide (NO)/pH/free  $Ca^{2+}$  of guard cells, while promoting the efflux of  $K^+$  and anions. A marked increase in the levels of NO as well as ROS occurs in guard cells of several species, like *Arabidopsis*, *Vicia* and pea, on exposure to ABA or methyl jasmonate or even microbial elicitors (e.g., chitosan). Real-time monitoring of ROS and NO in guard cells by fluorescent dyes, indicated that ROS acted upstream of the NO during stomatal closure. Levels of NO in guard cells plays a key role as a secondary messenger during stomatal closure induced by hormones as well as microbial elicitors. We have been studying the signaling events initiated by ABA as well as microbial elicitors, using the epidermis of pea or *Arabidopsis*. Our observations on the patterns of signaling components of guard cells would be presented.

The possible sources of NO and the cross-talk between NO and other signaling components would be discussed. A tentative scheme of events during stomatal closure by elicitors, highlighting the steps of divergence and convergence in signaling components presented.

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## **Abstract 6.6.**

# **INVESTIGATION OF ROOT SIGNALING UNDER HETEROGENEOUS SALT STRESS: A CASE STUDY FOR *CUCUMIS SATIVUS***

**Redwan M, Spinelli F, Masi E, Marti L, Bazihizina N, Azzarello E, Mancuso S**

To sense, respond and adapt to the constantly changing environmental conditions and in order to survive, plants have developed sophisticated signaling mechanisms. The aim of this study is to investigate plant signaling under heterogeneous salt conditions. A split root system was established and one half root apparatus (HR1) was treated with salt. The impact of the salt on the electrical signals as the initial responses of the plant to exterior stimulus was measured with a Multi Electrode Array (MEA) system in the non-stressed half roots (HR2). Both duration and amplitude of the action potentials recorded increased, while in the control remains similar. In order to identify the nature of the signal traveling from one HR1 to HR2, H<sup>+</sup> and K<sup>+</sup> fluxes were measured in HR2 by using ion-selective microelectrodes with the vibrating probe technique. A net potassium influx was observed after 40 minutes of the treatment while no change in proton flux was detected. The increased potassium concentration in HR2 was also confirmed by fluorescent dye potassium-binding benzofuran isophthalate (PBFI) compared with the control roots. By corona sodium green (fluorescent dye) and confocal microscopy we confirmed that changes in electrical signals and fluxes are not associated with the simple apoplastic diffusion of sodium from HR1 to HR2.

To further investigate the transmission of signals among roots, the activation of salt stress marker genes and expression level of ionic channel associated with K<sup>+</sup> homeostasis (e.g. AKT2) was examined in the split roots system. Potassium channels expression was not modulated in response to the treatment, as expected, since this gene is strictly regulated in leaves and not in the root. On the contrary the salt marker genes probed were upregulated in both portions of the root system after 3 and 4 h of the treatment with salt.

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## Abstract 6.7.

# INTERACTION BETWEEN *MEDICAGO TRUNCATULA* AND THE PATHOGENIC OOMYCETE *APHANOMYCES EUTEICHES* : EFFECTS OF NITROGEN NUTRITION AND PLANT GENOTYPE.

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Wendehenne D<sup>1,4</sup>, Truong HN<sup>2,4</sup> and  
Jeandroz S<sup>3,4</sup>

Plants are under the constant threat of microbial pathogens. To defend themselves, plants have developed immune responses (including for example synthesis of antimicrobial secondary metabolites, production of PR proteins or reinforcement of cell wall) that can lead to resistance. However, these plant defense responses are costly and lead plants to continuously face a dilemma regarding the partitioning of their available resources. In order to better understand relationships between plant nutrition and defense, we analyzed the impact of nitrogen (N) nutrition on the capacity of different *M. truncatula* genotypes to resist against *A. euteiches*, the causal agent of legume root rot disease. N stands at a crossroad between primary metabolism and defense and we consider that channeling of N metabolism represents a key point in the trade-off between plant growth and immune response. Two nutrition conditions and ten representative plant genotypes have been studied using an *in vitro* inoculation assay. Plants were phenotyped according to several macroscopic or molecular parameters already described as relevant indicators of plant resistance and principal component analyses were performed in order to estimate the behavior of the different genotypes.

First results have shown effects of genotype and nutrition taken individually, and interestingly strong interactions between genotypes and nutrition conditions. Indeed, for the F83005.5 genotype, sensitivity to *A. euteiches* increases under nitrate deficiency. In contrast, the tolerant A17 genotype, appears more resistant in the same condition. Experiments are in progress to investigate the molecular and biochemical processes underlying the expression of the N-dependent plant resistance. Special attention is given to the interaction between N nutrition, the production and role of Reactive Nitrogen Species such as nitric oxide (NO) and the expression of genes encoding proteins involved in N utilization and in secondary metabolism.

Keywords: *Medicago truncatula*, *Aphanomyces euteiches*, génotype, nutrition, nitrogen, plant immune response.

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# SESSION

## 7

### **BENEFICIAL TO PATHOGENIC PLANT MICROBE INTERACTIONS**

#### **Chairman Rafik Errakhi**

Plants are constantly subjected to various microbes which could be deleterious or beneficial. Plant-microbe interactions involved complex communication and signalization pathways which are not only crucial for better understanding plant growth and health, but could also be useful for sustainable crop production in a changing world. This session present some of the most recent advances in understanding plant-microbe interactions from beneficial interactions to symbiosis and pathogen interactions. A point of view on bio-products and the regulation of their uses for crops will be presented at the end of the session.

## Abstract 7.1.

# PRIMING WITH 2-AMINO-*N*-(2-AMINO- 3-PHENYLPROPANOYL)- *N*-HYDROXY-3- PHENYLPROPANAMIDE, A NOVEL HYDROXAMIC ACID-CONTAINING MOLECULE, CIRCUMVENT DEOXYNIVALENOL MYCOTOXIN -TRIGGERED EVENTS THAT LEAD TO CELL DEATH

**Yekkour A, Tran D, Arbelet-Bonnin D,  
Lebrihi A, Errakhi R, Sabaou N, Zitouni  
A, Bouteau F**

*Fusarium culmorum* and *F. graminearum* are responsible for devastating disease (Fusarium blights) that causes extensive yield and quality losses to cereals such as wheat and barley. These pathogenic fungi produce a wide range of mycotoxins, amongst which the deoxynivalenol (DON), a type B trichothecen believed to play a key role in the pathogenesis and disease aggressiveness. In the present context of fungicides limited effectiveness and controversy surrounding their applying, biocontrol of Fusarium-related blights using members of the *Streptomyces* genus already appeared as a prospective alternative with regard to the great ability of these microorganisms to produce a wide range of active substances. However, little is known about the *Streptomyces* derived compounds triggered plant-cell responses, which putatively permit to interfere with fungal effectors-triggered damages to the host. Through a pot experiment, we first evaluated the ability of a newly described hydroxamic acid-containing molecule [2-amino-*N*-(2-amino-3-phenylpropanoyl)-*N*-hydroxy-3-

phenylpropanamide, named W9], derived from a Saharan soil-living *Streptomyces* strain, to control *F. culmorum* seedling blight of barley. Seedling pretreatment with compound W9 permitted to significantly reduce both the disease occurrence on plant and the extent of blight symptoms by more than 75%. Subsequently, using cultured cells of *Nicotiana tabacum* BY-2, we have studied the effect of compound W9 on the early DON-induced cell-signaling events that lead to cell death. We showed that W9 could reduce the DON-induced programmed cell death (PCD) in BY-2 and the associated cell shrinkage. The clearly established reactive oxygen species (ROS)-dependent cell death triggered by the DON was significantly reduced after the addition of W9 as well as the DON-induced mitochondrial disturbance. Recovery of the DON-induced decrease in alternative oxidase activity was also evidenced.

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## Abstract 7.2.

# UNDER FUNGAL ATTACK ON A METALLIFEROUS SOIL: ROS OR NOT ROS? INSIGHTS FROM *SILENE PARADOXA* L. GROWING ON COPPER EXCESS.

**Colzi I<sup>a</sup>, Taiti C<sup>a</sup>, Giorni E<sup>b</sup>, Pignattelli S<sup>b</sup>, Bazihizina N<sup>a</sup>, Buccianti A<sup>c</sup>, Luti S<sup>d</sup>, Pazzagli L<sup>d</sup>, Gonnelli C<sup>b</sup> and Mancuso S<sup>a</sup>**

This work was performed to investigate if the adaptation to a metalliferous environment may have influence on the plant response to a biotic stress. In particular, we tested if the ability of the excluder metallophyte *Silene paradoxa* L. to cope with copper-induced oxidative stress could affect its behavior to pathogen-induced oxidative stress and its related responses. Previously, in comparison to a non-metalliferous population, an over-production of inducible defenses, precisely phytoalexins, was recorded for a copper mine population of *S. paradoxa* grown in the presence of copper and exposed to the PAMP (pathogen-associated molecular patterns) fungal protein cerato-platanin. In the present work, plants of the two contrasting populations were cultivated in the presence of copper and cerato-platanin, separately and in combination, to evaluate the induction of oxidative stress and the production of callose and volatiles. Our results evidenced that in the tolerant population the presence of copper in the medium seemed to prevent a normal PAMP-induced oxidative burst. Moreover, such effect was already present in control conditions, thus suggesting a constitutive incompatibility between the adaptation to metalliferous environments and a "ordinary" ROS-mediated plant defense response, as it was postulated for hyperaccumulating plants. Different ways of signaling the pathogen attack could be hypothesized

for the metallicolous population, probably through altered salicylic/jasmonic acid pathways. When grown in presence of copper, a similar situation of modified PAMP-induced oxidative burst was showed also by the sensitive population, but in this case also with the lack of some responses, such as the production of callose. In addition, in terms of joint behavior of emitted VOCs, multivariate statistics showed that not only the populations seemed to behave in a different way in presence of copper or biotic stress, but also that the biotic and abiotic stresses interacted in different ways in the two populations.

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## Abstract 7.3.

# INDUCTION OF PLANT DEFENSE AND RESISTANCE BY *GAULTHERIA PROCUMBENS* ESSENTIAL OIL, A NATURAL SOURCE OF METHYLSALICYLATE

**Dumas B<sup>1</sup>, Vergnes S<sup>1</sup>, Fournier S<sup>1</sup>, Ladouce N<sup>1</sup>, Attia F<sup>3</sup>**

Essential oil from *Gaultheria procumbens* mainly composed of methylsalicylate (>99%), a compound which can be metabolized in plant tissues to salicylic acid, a phytohormone inducing plant immunity against microbial pathogens. The potential use of *G. procumbens* essential oil as a biocontrol agent was evaluated on the model plant *Arabidopsis thaliana*. Expression of a selection of defense genes was detected 1, 6 and 24 hours after essential oil treatment (0.1 ml/L) using a high-throughput qPCR-based microfluidic technology. Control treatments included methyl jasmonate and a commercialized salicylic acid analog, acibenzolar-S-méthyl (BION). Strong induction of defense markers known to be regulated by the salicylic acid pathway was observed after the treatment with *G. procumbens* essential oil. Treatment induced the accumulation of total salicylic acid in the wild -type *Arabidopsis* line Col-0 and analysis of the *Arabidopsis* line *sid2*, mutated in a salicylic acid biosynthetic gene, revealed that approximately 30% of methylsalicylate sprayed on the leaves penetrated inside plant tissues and was demethylated by endogenous esterases. Induction of plant resistance by *G. procumbens* essential oil was tested following inoculation with a GFP-expressing strain of the *Arabidopsis* fungal pathogen *Colletotric humhigginsianum*.

Fluorescence measurement of infected tissues revealed that the treatment led to a strong reduction (70%) of pathogen development and that the efficacy of the *G. procumbens* essential oil was similar to the commercial product BION. Together, these results show that the *G. procumbens* essential oil is a natural source of methylsalicylate which can be formulated to develop new biocontrol products.

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## Abstract 7.4.

# NO SIGNALING IN TOBACCO ELICITED BY THE MAMP CRYPTOGEIN

**Lamotte O<sup>1</sup>, Aimé S<sup>4</sup>, Rosnoblet C<sup>2</sup>,  
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During the past years, nitric oxide (NO) has been shown to be a major cell signaling messenger in plants. Its importance has been highlighted during plant responses to pathogen attack or MAMPs (microbe associated molecular patterns) and during induced resistance or priming phenomenon. The major focus of our research is to understand how nitric oxide can modulate the activity of protein involved in plant defense. We identified several proteins undergoing S-nitrosylation, a redox-based post-translational modification of proteins, in tobacco cells elicited by cryptogein, a 10 kDa protein produced by the oomycete *Phytophthora cryptogea* and inducing immune responses in tobacco. These proteins include the chaperone-like AAA+-ATPase CDC48 and a calmodulin isoform. The incidence of NO on the structure and function of both proteins has been investigated. Concerning CDC48, the S-nitrosylated cystein is located in the vicinity of the ATP binding site in the second ATPase domain. Its S-nitrosylation triggers local structural changes that correlates with a strong reduction of the ATPase activity. Calmodulins are Ca<sup>2+</sup> sensors that decode Ca<sup>2+</sup> signals in all eukaryotes organisms. Ca<sup>2+</sup> binds to 4 EF-hand domains inducing a conformational change of the protein. This change is required for CaM binding to target protein then modulating their activities. We showed that a recombinant CaM is S-nitrosylated on a unique cystein residue either in the presence or in the absence of Ca<sup>2+</sup>. This cystein residue is located in the first EF-hand domain suggesting a role of

the S-nitrosylation in CaM function. Through the identification of new components of NO signaling, our work further supports the concept that NO is a key mediator in the signaling cascade leading to immune responses in plants.

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## Abstract 7.5.

# ENVIRONMENTAL STRESS TOLERANCE POTENTIAL OF TRANSGENIC MINT WITH ENHANCED GSH CONTENT

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Mint leaves serve as the main source of menthol, is widely used in food, flavor, cosmetic and pharmaceutical industries. A major constraint to menthol production is the disease caused by a variety of fungal pathogens such as *Puccinia menthae*, *Verticillium dahliae*, *Verticillium albo-atrum*, *Phoma strasseri*, *Erysiphe cichoracearum*, *Alternaria alternata* and *Rhizoctonia solani*. Various environmental stresses like metal toxicity also caused extensive damage to this crop. Here, an approach has been taken to raise transgenic mint over-expressing  $\gamma$ -glutamyl-cysteine synthetase ( $\gamma$ -ECS), the rate-limiting enzyme of GSH biosynthesis, resulted enhanced GSH content. Transgenic mint also confers significant tolerance towards abiotic and biotic stresses viz. metal toxicity – Cd, Zn as well as *A. alternata* and *R. solani*. Furthermore, to identify the target proteins from transgenic mint under a necrotrophic fungus, *A. alternata* infected condition; a 2-DE approach followed by MALDI TOF-TOF MSMS analysis was performed. Detection of various proteins/TFs viz. disease resistance proteins, thioredoxin proteins, GST, WRKY-71, MAP kinase like protein, ERK2 etc. clearly point towards the involvement of GSH through downstream defense/redox regulated and signaling pathways in environmental stress tolerance. The results demonstrate that transgenic crops with enhanced GSH content may be an effective and versatile system to protect economically important crops against natural environmental stresses.

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## Abstract 7.6.

# CYTOKININ AND AUXIN REGULATION OF LATE STAGES OF PEA (*PISUM SATIVUM* L.) SYMBIOTIC NODULE DEVELOPMENT

**Tsyganova AV, Kusakin PG,**  
**Tsyganov VE**

Immunocytochemical analysis of cytokinin and IAA localization in pea wild-type and symbiotic mutant nodules was performed. Fluorescent microscopy showed that in nodules of the wild-type line SGE the maximum concentration of cytokinin was observed in the infection and nitrogen-fixation zones. In contrast, in the mutant SGEFix-1 (*sym40*), it was observed in the meristem. Ultrastructural analysis of wild-type nodules revealed that cytokinins were accumulated in the infection thread matrix, the peribacteroid space, plastids and cell walls. In the mutant SGEFix-1 the amount of label was significantly decreased. Previously, it was suggested that the fine balance of cytokinins in different zones of the developing nodule may be important for cell differentiation. The mutant SGEFix-1 is characterized with an abnormal histological organization of the nodule. In *Medicago truncatula*, the orthologue of the pea gene *Sym40* encodes the transcription factor EFD, which activates a negative regulator of the cytokinin response *RR4*. Thus, abnormalities in the regulation of the cytokinin response associated with mutation *sym40* may determine abnormalities in cytokinin localization in the mutant nodule: as a consequence, this could lead to abnormal nodule histological organization. Fluorescent microscopy revealed IAA in the meristem and peripheral tissues in wild-type and mutant nodules.

Ultrastructural analysis showed that in wild-type and mutant nodules IAA is localized preferentially in the nuclei of infected cells and in the cytoplasm. At the same time, IAA was practically absent from the infection thread matrix, but was present in bacteria and peribacteroid space. In the mutant RisFixV (*sym42*), IAA was observed in thickened infection thread walls, enriched with unesterified pectin and callose. Thus, in the mutant RisFixV, IAA is involved in the process of pectin de-esterification, an aspect of the host defense reaction that is associated with fortification of the infection thread wall.

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## **Abstract 7.7.**

# **BIOPRODUCTS UNDER THE REGULATION POINT OF VIEW,**

## **Grosbeau K**

The current societal and regulatory context is favourable to bioproducts market. Bioproducts include biostimulants and biocontrol products also called biopesticides. Biostimulants and biocontrol products have different beneficial effects to plants, their definition is now well established.

In Europe, their regulation is handled differently, whereas the regulation of biocontrol products is already in place as they are Plant Protection Products, these of the biostimulant is not yet set up. Biostimulants will be included in the Fertiliser Regulation and until its entry into force national legislations of the EU Members States need to be applied.

In Europe, some measures have been taken which are considered as favourable to biocontrol products and biostimulants, First the Directive 2009/128/EC, the Sustainable Use of Pesticides Directive, and its implementation, and the related National Action Plans for the Risk and/or Reduction of use of pesticides.

Then in the Regulation N°1107/2009 which rules the placing on the EU market Plant Protection Products, 2 articles are in favor of biocontrol products; they concern the low risk substances and candidate for substitutions topics.

The placing on the market of products containing these low risk substances will be facilitated by shorter timelines and costs. On another hand, products containing active substances listed as candidate for substitution, will be subjected to a comparative assessment measure by Member States before being placed on the market.

The researches on alternative solutions for agriculture are under expansion since years, but it took times for the regulators to adapt the legislations to these products. Member States have now the tools to sustain the placing on the market of biocontrol products and it will be the case also for biostimulant in a close future.

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# SESSION

## 8

### PHYTOHORMONES AND SIGNALING

**Chairman Axel Mithöfer**

Many processes in plants' development as well as their interactions with the biotic and abiotic environment needs strict coordination and regulation of appropriate molecular, biochemical and physiological cellular reactions. This means intricate and complex pathways of both signal perception and transduction are very likely employed to allow optimal responses. Signal recognition and transduction in cells involves specific signaling molecules such as intracellular calcium, inositolphosphates and various types of phytohormones, including abscisic acid, auxin, gibberellins, ethylene, jasmonates, salicylic acid, cytokinins, which mediate the action of particular signaling pathways. The molecular mechanisms involved in such processes have been revealed comparatively independently. However, recent studies showed several principles and molecules that are common players in individual as well as in crosstalk between signaling pathways. In this session various aspects and novel findings in phytohormone- and other signaling compounds-regulated processes will be presented and discussed.

## Abstract 8.1.

# UNDERSTANDING FLORAL TRANSITION AT THE SHOOT APICAL MERISTEM OF SOYBEAN

**Bhalla PL, Wong CE, Jung CH, Liew LC,  
Singh MB**

Flowering process governs seed set and hence influences crop productivity. Understanding of the floral transition is vital in ensuring future food security under changing climate. Soybean [*Glycine max* (L.) Merr.] is one of the world's most important crops, being responsible for 55% of the worldwide oilseed production and has the capacity to fix atmospheric nitrogen further enhances its significance in the world agriculture. Soybean is a photoperiod sensitive crop whose floral transition is triggered by exposure to short-day conditions. Our understanding of the molecular control of flower initiation in this economically important legume species is limited. We used an integrated bioinformatic and experimental approach to address this gap in our knowledge. Our comparative genomics study revealed complete repertoire of flowering regulatory genes in the soybean genome. Further, RNA-Seq analysis of shoot apical meristem and leaf undergoing floral transition revealed major reprogramming events in leaf and the shoot apical meristem that point toward hormones gibberellins and cytokinin as key regulators in the production of systemic flowering signal(s) in leaves.

Our data also revealed an extensive reprogramming of genes associated with the epigenetic chromatin modifications and RNAi gene silencing in the shoot apical meristem during floral transition. Our results highlight the utility of a genomics-based approach in enhancing our understanding of this important developmental process and have provided a framework to investigate further the molecular network underlying the transition to flowering in soybean.

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## **Abstract 8.2.**

# **QUESTIONING THE CLASSICAL RELEVANCE OF AUXIN FOR MAIZE ROOT GROWTH REGULATION; - TIME FOR A NEW ROOT GROWTH REGULATORY MODEL?**

**Edelmann HG, Hahn A**

From the very beginnings of auxin (IAA) research exogenous application to either IAA-depleted organ segments –i.e. of coleoptiles or hypocotyls –but also intact systems such as shoots or roots, served for decades as the basis experiment for the elucidation of its growth promoting mechanism. Employing this classical experimental procedure we recapitulated previous observations concerning the apparent opposite effects of IAA on shoot- as well as on root growth. Applied via defined incubation-solutions as well as pastes, IAA promoted elongation growth of shoot segments and coleoptiles respectively. Also inhibition of root growth was affected in a concentration dependent manner, when roots were incubated in appropriate solutions, a procedure identical to the bulk of previous root growth studies. However, the very same solutions and pastes had no effect on neither elongation growth of roots nor on gravitropic differential growth, as long as the root cap was unaffected/intact. Root elongation growth during 24 h and more showed no difference between IAA-solution- and water- incubations, the volume-increase of which was accomplished via the solution-imbibed shoot part of the seedlings–although secondary root formation was strongly affected by IAA. This indicates that the often reiterated inhibiting effect on root growth acts via a mechanism originating from the root cap, dependent on IAA, which itself, however, has no effect on elongation growth of the cells of the root proper.

By use of the currently most sensitive detection methods [ETD300], ongoing analyses strongly imply ethylene to play a so far unknown major elongation regulating role of the root cells – in dependence of IAA.

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## Abstract 8.3.

# INOSITOL POLYPHOSPHATES AS FUNCTIONAL COFACTORS OF F-BOX PROTEINS INVOLVED IN THE PERCEPTION OF AUXIN AND JASMONATE

Heinz T<sup>1</sup>, Werner S<sup>1</sup>,  
CalderónVillalobos LIA<sup>2</sup>, Heilmann I<sup>1</sup>

The phytohormones, auxin and jasmonate, are perceived by a type of ubiquitin-ligase complexes that mediate ubiquitylation and proteasomal degradation of transcriptional repressors. Specifically, the phytohormones bind to leucine rich repeat (LRR) F-box proteins (FBPs) which recruit the degradation targets into ubiquitin-ligase complexes in a phytohormone-dependent manner. The structural elucidation of the FBPs, TIR1 and COI1, involved in the perception of auxin and jasmonate-isoleucine respectively, indicates the presence of inositolpolyphosphates (IPPs) as tightly bound cofactors in these proteins (inositolhexakisphosphate, IP<sub>6</sub>, in TIR1 and inositolpentakisphosphate, IP<sub>5</sub>, in COI1). It is our goal to determine whether or not these cofactors have relevance for the function of the FBPs and for phytohormone perception. A set of transgenic Arabidopsis lines was established in which the levels of IPPs are modulated to differ from the wild type situation. These plant lines are systematically analyzed for cellular responses involving auxin and jasmonate, and for their responses to exogenously applied phytohormones. The tests address physiological read-outs, such as gravitropic bending, defence against herbivorous caterpillars or root growth inhibition, as well as gene expression analyses and indicate that IPPs are functional cofactors of LRR FBPs involved in the perception of auxin or jasmonate-isoleucine.

We are currently contending the notion that the interconversion of IP<sub>5</sub> and IP<sub>6</sub>, which is mediated by one enzymatic step, might be an important event in the switch between auxin-mediated growth to jasmonate-isoleucine-mediated defence responses. This work is supported by the German Research Foundation (DFG, grant He34214/2-2 and CRC648 TP B13, to IH).

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## Abstract 8.4.

# HOW C3 PLANTS BALANCE COMPETING PRIORITIES BETWEEN H<sub>2</sub>O AND CO<sub>2</sub>

**Leung J<sup>1</sup>, Leonhardt N, Tran D, Véry  
AV, Bouteau F, Jammes F**

Sensing declining soil-water potential, plants will accumulate abscisic acid (ABA), which then triggers stomatal closure to conserve tissue moisture. Prolonged stomatal closure, however, creates a dilemma in that it will cause a shortage of CO<sub>2</sub> for photosynthesis. In *Arabidopsis thaliana*, CO<sub>2</sub> depletion will in turn suppress ABA sensitivity, (re)opening stomates despite the increased risk of severe water loss. Resolving this H<sub>2</sub>O-CO<sub>2</sub> conflicting priorities is critical for plants' long-term survival, as attested by different strategies that have evolved in nature. The most familiar examples are high-affinity CO<sub>2</sub> fixation by PEPC (C4 carbon fixation), uncoupled photosynthesis (day) from CO<sub>2</sub> fixation (night) (CAM), or to suspend animation, thus dispensing with the need for CO<sub>2</sub> (poikilohydric). In contrast to these examples, typical C3 plants like *Arabidopsis* thrive rather in temperate geoclimatic zones, generally with abundant soil water and high atmospheric humidity. Still, failure to continuously adjusting to small changes in regional H<sub>2</sub>O supply and CO<sub>2</sub> demands will eventually exact a fitness cost to plants under natural selection. How C3 plants balance the competing priorities between H<sub>2</sub>O and CO<sub>2</sub> is not understood. We explored its molecular basis by designing an experimental stress system in which *Arabidopsis* is confronted with this conflicting needs for H<sub>2</sub>O-CO<sub>2</sub>. This led us to identify a latent anti-abscisic acid metabolite, 1,3-diaminopropane (DAP), whose activity requires its switching on by an acetyltransferase, NATA1.

Acetylated DAP can regulate a host of plasma membrane ion transport activities and electrical properties opposite in directions to the same ones by ABA. Acetyl-DAP is thus a metabolite switch, and along with ABA, ease stomatal apertures reversibly in continuum with changing soil-water availability to balance both tissue humidity and the demands for photosynthetic CO<sub>2</sub>.

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## Abstract 8.5.

# VISUALIZATION OF SYSTEMIC CYTOSOLIC $\text{Ca}^{2+}$ ELEVATION UPON WOUNDING AND HERBIVORY IN *ARABIDOPSIS THALIANA*

**Mithöfer A, Kiep V, Vadassery J and Peiter E**

In higher plants,  $[\text{Ca}^{2+}]_{\text{cyt}}$  signals play an important role in the regulation of stress-related responses. Recent studies indicated that intracellular  $\text{Ca}^{2+}$  signalling triggered by insect herbivory is an intricate network with multiple components, involving positive and negative regulators of appropriate defense reactions. In order to visualize the induced  $[\text{Ca}^{2+}]_{\text{cyt}}$  signals, real-time, non-invasive imaging of entire *Arabidopsis thaliana* rosettes- expressing the cytosol-localized  $\text{Ca}^{2+}$  reporter aequorin - was performed using a high-resolution photon-counting camera system. I will present results providing evidence that in *Arabidopsis* insect herbivory induces both local and systemic  $[\text{Ca}^{2+}]_{\text{cyt}}$  signals that distribute within the vascular system. In more detail, cytosolic free calcium  $[[\text{Ca}^{2+}]_{\text{cyt}}]$  elevations in both local and systemic leaves were monitored in response to wounding and *Spodoptera littoralis* feeding. *S. littoralis* feeding on *Arabidopsis* induced both local and systemic  $[\text{Ca}^{2+}]_{\text{cyt}}$  elevations. Systemic  $[\text{Ca}^{2+}]_{\text{cyt}}$  signals were found predominantly in adjacent leaves with direct vascular connections to the treated leaf and appeared with a delay of 1 to 2 minutes. Simulated herbivory by wounding always induced a local  $[\text{Ca}^{2+}]_{\text{cyt}}$  response, but a systemic one only when the midrib was wounded.

This systemic  $[\text{Ca}^{2+}]_{\text{cyt}}$  response was suppressed by the presence of insect-derived oral secretions as well as in a mutant of the vacuolar cation channel TPC1. The underlying mechanisms of systemic herbivory-induced signalling will be discussed.

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## Abstract 8.6.

# UNDERSTANDING OF FREE JA AND ME-JA SIGNALLING CASCADE IN *CAJANUS CAJAN* DURING COPPER STRESS

**Sirhindi G, Sharma P, and Singh KA<sup>1</sup>**

*Cajanus cajan* subjected to 5 mM CuSO<sub>4</sub> exhibit decrease in seed germination and elongation growth of shoot and root while dynamism in fresh and dry weight in 15 day old seedlings was observed. Jasmonates is a group of oxylipin is well known signalling molecule resist plants from various abiotic and biotic stresses. In present study two signal cascades have been investigated which could be followed by two derivatives of JA viz. free-JA and methyl ester of JA (Me-JA) with or without Cu stress in *C. cajan*. From the gene expression MAPK kinase and Ca<sup>2+</sup> - CAM cascades it has been suggested that two derivatives of JA used in present work, followed independent signalling pathway to give the same tolerance response in terms of antioxidant enzymes: SOD and CAT along with up-regulation of PR gene expressions. The result proposed that Me-JA triggered both MAPK Kinases and Ca<sup>2+</sup> - CAM signal cascade to up-regulate antioxidant enzymes and PR gene expression make plant Cu tolerant. Though free JA not follow the same pathway consequently shows that free JA might have another independent cascade to follow which yet to be explored. Treatment of free-JA and Me-JA down-regulate the expression of COI1 and MYC2 genes which are the major players in JA-Ile signalling to make plants tolerant to abiotic stress.

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## Abstract 8.7.

# ONE WAY TO ACHIEVE GERMINATION: COMMON MOLECULAR MECHANISM OF ETHYLENE AND AFTER- RIPENING

**Xia Q, Pellen M, Gilard F, Perreau F,  
Huguet S, Balzergue S, Langlade N,  
Bailly C, El-Maarouf-Bouteau H**

Seed dormancy is defined as the inability to germinate at favorable conditions. Dormancy alleviates during after-ripening, a dry storage period, depending on environmental conditions such as temperature, light or oxygen. Treatment of dormant seeds (D) with ethylene promotes seed germination and ABA treatment inhibits non-dormant seed germination in sunflower, *Helianthus annuus*. Metabolomic and transcriptomic studies have been performed during imbibition to compare germinating seeds, ND and D treated with ethylene (D/Eth) and non-germinating seeds D and ND treated with ABA (ND/ABA). PCA analysis of metabolites quantification showed that imbibition did not trigger significant change in the first hours. Metabolic change associated to germination capacity occurred at 24h and was related to hexoses, as their content was higher in ND and D/Eth and reduced by ABA treatment. At the transcriptional level, a large number of genes were altered oppositely in germinating as compared to non-germinating seeds. Metabolomic and transcriptomic results were integrated in the interpretation of processes involved in germination. Our results show that ethylene treatment triggers molecular change comparable to that of after-ripening treatment concerning sugar metabolism and ABA signaling inhibition.

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## Abstract 8.8.

# HORMONAL AND ENVIRONMENTAL REGULATIONS OF SEED DORMANCY AND GERMINATION IN ARABIDOPSIS

**Yan D and Nambara E**

Seeds respond to multiple different environmental stimuli and these environmental conditions in turn regulate seed dormancy and germination. It is a challenge to understand how multiple different signals coordinately regulate a common downstream event, like germination. To this end, it is necessary to identify the common regulators of germination and elucidate how individual environmental signaling pathway regulates the common regulators. Plant hormone metabolism and signaling are good candidates for such common regulators. Abscisic acid (ABA), gibberellins (GAs), ethylene and other plant hormones are involved in the germination control. ABA is a germination inhibitor that accumulates in dry seeds, and the reduction of ABA levels in imbibed seeds is a prerequisite for successful seed germination. In *Arabidopsis*, an ABA catabolic gene, *CYP707A2*, plays an essential role in the germination control through reducing seed ABA contents in response to environmental changes. In the meeting, we will present our recent results concerning how nitrate regulates the expression of *CYP707A2* during seed germination.

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# SESSION

## 9

### DEVELOPMENTAL BIOLOGY

#### Chairman Arnaud Lehner

The session “developmental biology” will host six researchers coming from 6 different countries and working on two models: the pollen tube growth and the root growth. The session will begin with the pollen tube, a single cell, with a tip polarized growth which is often used as a model to study the cell wall biosynthesis. In the first talk, A. Lehner will focus on pollen tubes and vegetative organs from Solanaceae plants and on their cell wall specificities, particularly regarding the xyloglucan, whereas during the second talk, A. Boisson-Dernier will present his work on the cell wall-sensing receptors that regulate Reactive Oxygen Species production, Calcium homeostasis and exocytosis in order to coordinate and to regulate the cell wall performances and properties. The session will then move below the ground and the main subject will become the root, its development and the regulation of its growth. First, S. Yalosky will talk about the remarkable phytohormone auxin and the involvement of auxin-induced  $\text{Ca}^{2+}$  binding protein in microtubule destabilisation and in auxin gradient formation.

The presentation of PH. Tournier which presents his work on the use of mathematical and numerical tools to develop explicit mechanistic models that simulate soil water and solute movement at the whole root system scale. Finally M Staves and H Susuzuki will discuss the impact of light on plant gravity sensing.

## Abstract 9.1.

# POLLEN TUBES AND VEGETATIVE ORGANS HAVE STRUCTURALLY DIVERGENT CELL WALL XYLOGLUCAN IN SOLANACEAE SPECIES.

**Dardelle<sup>1</sup> F, Le Mauff<sup>1</sup> F, Loutelier-Bourhis<sup>2</sup> C, Bardor<sup>1</sup> M, Rihouey<sup>3</sup> C, Causse<sup>4</sup> M, Lerouge<sup>1</sup> P, Driouich<sup>1</sup> A, Mollet<sup>1</sup> JC, and Lehner A<sup>1</sup>**

Fertilization in plant relies on the proper targeting of the ovules by the pollen tube, a fast growing tip-polarized cell. During growth, a massive vesicle secretion promotes the proper assembly of the pollen tube cell wall that plays a capital role on the growth dynamic, mechanical properties and presumably guidance by interaction with the female tissues. One of the cell wall polymers, typically xyloglucan (XyG) in most eudicots, is known for maintaining cell wall integrity and allowing cell expansion. We previously reported on *Arabidopsis thaliana* pollen tube cell wall that XyG were highly fucosylated and *O*-acetylated compared to the one found in vegetative organs, suggesting an important role of fucosylation and acetylation of XyG in pollen tube growth. Interestingly, the XyG contained in vegetative organs and cell suspension of plants from the clade Asterids (Eudicot including the Solanaceae) lacks the fucosyl residues and instead exhibits an arabinoXyG, due presumably to an adaptative and/or selective diversification. Here, we show that *Nicotiana tabacum* and *Solanum lycopersicum* (Solanaceae) pollen tubes contained fucosylated XyG. Thus, pollen tubes and vegetative organs from Solanaceae plants have structurally divergent XyG suggesting that the pollen tubes have a specific set of functional biosynthetic enzymes. The presence of fucosylated XyG in the pollen tubes from Arabidopsis (Malvid,

Brassicaceae) and tobacco/tomato (Asterids, Solanaceae) also suggests that the male gametophyte is more conservative and it has not evolved at the same pace than the vegetative sporophyte. Thus, fucosyl residues of XyG may play an important role in this capital step of plant reproduction resulting in seed production.

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## Abstract 9.2.

# TO BURST OR NOT TO BURST: A TALE OF RECEPTOR-LIKE KINASES IN POLLEN TUBE.

**Franck CM, Lituiev D, Grossniklaus U  
and Boisson-Dernier A**

It has become increasingly apparent that the plant cell wall (CW) can influence intracellular activities in ways that go far beyond its supposedly passive mechanical support. Plant growing cells use mechanisms sensing CW integrity to coordinate CW performance with the internal growth machinery to avoid growth cessation or loss of integrity. How this coordination precisely works is unknown. Previously, we reported that in the tip-growing pollen tube the ANXUR receptor-like kinases (RLKs) of the CrRLK1L subfamily are essential to sustain growth without loss of CW integrity. Over-expression of the ANXUR RLKs led to over-activation of exocytosis and the over-accumulation of secreted membrane and CW material that subsequently triggered growth arrest. Moreover, the characterization of T-DNA insertions in two partially redundant pollen-expressed NADPH oxidases coupled with genetic interaction studies demonstrate that the ANXUR RLKs function upstream of these NADPH oxidases. Using genetically-encoded ratiometric sensors in NADPH oxidase-deficient mutants, we reveal that NADPH oxidases generate a tip-localized, pulsating  $H_2O_2$  production that possibly functions to maintain a steady tip-focused  $Ca^{2+}$  gradient during growth. Our findings support a model where CW-sensing receptors regulate ROS production,  $Ca^{2+}$  homeostasis and exocytosis to coordinate CW performance with the internal growth machinery. Moreover, to identify new players of this largely unexplored pathway, an *anaxur* sterility EMS-induced suppressor screen was performed and led to the identification

of suppressors with improved fertility due to rescue of *anaxur* PT growth. For two suppressors, mapping by next-generation sequencing identified non-synonymous mutations in key functional domains of a receptor-like cytoplasmic kinase (RLCK) and a protein Ser/Thr phosphatase (PPP). Both the RLCK and the PPP are pollen-preferentially expressed and were named MARIS (MRI) and ATUNIS1 (AUN1) according to Etruscan deities of fertility and rebirth, respectively. Advances in the genetic and molecular characterization of these new components of the CW integrity pathway will be presented.

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## Abstract 9.3.

# REGULATION OF AUXIN DISTRIBUTION BY A ROP EFFECTOR AND A $Ca^{2+}$ SENSOR DEPENDENT MICROTUBULES STABILITY SWITCH

**Hazak O, Lavy M, Sternberg H and Yalovsky S**

Pattern formation in plants depends on polar transport and formation of local maxima and gradients of the phytohormone auxin. Remarkably, auxin self regulates its own transport and accumulation, implying a tight link between auxin signaling and mechanisms that regulate its distribution. Previously, we have identified the ICR family of ROP (Rho of Plants) effectors and found that a member of the family, designated ICR1, is required for recruitment of PIN auxin efflux transporters to the plasma membrane. Remarkably, ICR1 expression is induced by auxin but it is degraded at the site of auxin maximum formation at the root tip. The degradation of ICR1 is induced by high auxin concentrations and depends on TIR1/AFB regulated auxin induced gene expression. We will show that ICR1 is a microtubules (MTs) binding protein. Mutant complementation assays indicate that MTs binding is required for ICR1 function. Through its interaction with ROPs, ICR1 recruits and stabilizes MTs in focal plasma membrane domains. The interaction of ICR1 with MTs is negatively regulated by an auxin-induced  $Ca^{2+}$  binding protein, called CMI1, which is highly expressed at the site of ICR1 degradation at the root meristem. Our data indicate that CMI1 is part of an auxin- $Ca^{2+}$  regulated feedback loop that leads to ICR1 degradation, MTs destabilization and local auxin maximum formation.

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## Abstract 9.4

# SHINING BLUE LIGHT ON THE MECHANISM OF PLANT GRAVITY SENSING

**Staves MP and Kyle B**

Since 1900 the most widely-accepted model for plant gravity sensing has been the starch-statolith model which proposes that sedimenting intracellular particles are the gravity sensors. A shortcoming of this model is that there are examples of gravity-responsive plants and plant tissues which do not contain sedimenting statoliths. We proposed an alternative model for plant gravity sensing (the gravitational pressure model) in which the entire protoplast is suggested to be the gravity sensor and that the gravity signal is perceived by sensing differential pressure between the protoplast and the extracellular matrix at the top and the bottom of the cell. To test between these models we grew rice roots in media of different densities and monitored gravity-induced curvature. If the protoplast were the gravity sensor, we predict that increasing the density of the external medium would decrease gravity-induced curvature. If statoliths were responsible for gravity sensing, we expect that changing the external medium with an impermeant solute would have no effect on the gravity response. Consistent with the gravitational pressure model, we find that increasing the density of the external medium inhibits gravity-induced curvature. However it is possible that the inhibition of gravity-induced curvature reflects growth inhibition rather than an inhibition of gravity sensing. To test this, we took advantage of the negative phototropism of roots exposed to blue light. Vertically-grown rice roots were illuminated with blue light perpendicular to the vector of gravity such that positively-gravitropic growth was antagonistic to negatively-phototropic growth.

The starch-statolith model predicts that changing the density of the external medium would have no effect on negative phototropism, while the gravitational pressure model predicts that increasing the density of the external medium will *increase* the negative phototropism (by inhibiting gravity sensing). Consistent with the gravitational pressure model, increasing density of the external medium increases negative phototropic curvature.

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## Abstract 9.5.

# LIGHT AND ROOT CAP-DEPENDENT GRAVITROPISM OF MAIZE ROOT REQUIRES AUXIN BIOSYNTHESIS

**Suzuki H<sup>1</sup>, Yokawa K<sup>1,2</sup>, Okamoto T<sup>1</sup>, Baluška F<sup>2</sup> and Koshiba T<sup>1</sup>**

Gravitropism is a plant response to determine the direction of shoot and root growth. Roots are well known to show positive gravitropism. Root cap is thought to be a site for sensing gravity, thus it is necessary for growth of root to the ground. Interestingly, some plants require light irradiation to root cap for exhibiting this tropic response. We confirmed roots of maize showed gravitropism in a light and root cap-dependent manner. When roots were exposed to white light (34.7  $\mu\text{mol}/\text{m}^2$ ) continuously at least 1-2 hours, we found increase of indole-3-acetic acid (IAA) levels in root tips, especially at the transition zone. Treatment of l-kynurenine and yucasin, inhibitors of IAA biosynthesis, inhibited not only the IAA increase but also root curvature under the light condition. These results indicated that the synthesis of IAA involves light-dependent gravitropic curvature of maize roots. We also demonstrated that IAA is synthesized from stable isotope labeled tryptophan ( $^{13}\text{C}^{15}\text{N-Trp}$ ) in the tip region of roots. Some *ZmYUC* genes, coding IAA biosynthesis enzymes, were expressed in the root tip, suggesting that IAA is biosynthesized via YUC pathway at the site. We further checked effects of auxin transport inhibitors, such as BFA, NPA, and 1-NOA, but these showed no clear effect on either amount of IAA or gravitropism. All this suggests the presence of the complicated IAA transport system in the root apex. In summary, our results indicate that de novo auxin biosynthesis induced by light irradiation is important for the root-cap dependent gravitropism in maize roots.

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## **Abstract 9.6.**

# **MATHEMATICAL MODELLING AND NUMERICAL SIMULATION OF WATER AND NUTRIENT UPTAKE BY PLANT ROOTS**

**Tournier PH**

In the context of the development of sustainable agriculture aiming at preserving natural resources and ecosystems, it is necessary to improve our understanding of underground processes and interactions between soil and plant roots. Although the root system is responsible for taking up water and nutrients from the surrounding soil, roots are hidden below ground and measurements are difficult to obtain.

In this work, we use mathematical and numerical tools to develop explicit mechanistic models governed by nonlinear partial differential equations that simulate soil water and solute movement with plant root uptake at the whole root system scale while taking the three-dimensional architecture of the root system explicitly into account. The numerical models take advantage of the recent advances of scientific computing in the field of unstructured mesh adaptation and parallel computing and are able to resolve the geometry of the root system as well as small scale processes occurring in the rhizosphere, which play a major role in plant root uptake. Such models can be used to study how the uptake pattern is affected by the shape and architecture of the root system. Numerical simulations of water, phosphate and nitrate uptake by maize root systems including root growth and showing the effects of hydrotropism and chemotropism illustrate the capabilities of the models.

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# SESSION 10

## **BROADER SIGNIFICANCE OF PLANT BEHAVIOR**

**Chairman Elisabeth Van Volkenburgh**

Plant behavior provokes thought beyond the biological sciences, into realms of anthropology, philosophy, policy and beyond. Possibly, the spectres of climate change and global over-population by humans is turning our focus in a positive way to plants as biological organisms. How do they work, what is their contribution to our well-being, and how do they influence us and our future? Plant biologists are called upon to share their work beyond their fields of research. This session will address how our findings in plant biology resonate with and interest scholars beyond biology, and how technology and policy decisions may reflect this change in comprehension of plant function.

## **Abstract 10.1.**

# **THE DIGNITY OF PLANTS**

## **Koechlin F**

The Swiss Constitution maintains that the dignity of creatures – including plants – must be respected. But what could this mean? What could be the consequences? Some ethical and practical considerations.

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## **Abstract 10.2.**

# **TOWARD AN ANTHROPOLOGY OF THE VEGETAL SENSORIUM: BETWEEN SIGNAL, SENSE, AND SENTIENCE**

## **Myers N**

Recent and remarkable findings in the field of plant signaling and behaviour have drawn the attention, not only of philosophers, artists, and popularizers of science, but also of anthropologists. Anthropology has a long history of engagement with natural sciences. Anthropologists work alongside scientists in their laboratories to explore science as a culture and as a practice, taking interest in the ways that distinct communities of researchers collectively shape new fields of inquiry, conduct their experiments, and adjudicate facts. With keen attention to language, and to the uses of metaphor and story, anthropologists can generate significant insights into cultural phenomena such as the ubiquitous use of anthropomorphism in scientific research. This paper shares anthropological findings from interviews with a range of plant signaling researchers in the US, Canada, and Europe, who are studying phenomena such as shade avoidance, the evolution and biomechanics of pollination, circadian rhythms, and chemical ecology. It offers insights into the challenges researchers confront working in “thought collectives” that simultaneously denounce and tacitly incorporate anthropomorphisms into their models of plant behaviour.

With the recognition that the use of anthropomorphism is widespread and integral to scientific thinking, writ large, the paper explores the promises and possibilities set in motion by metaphors that help researchers bridge the gap between the known and the unknown as they attempt to make sense of the relationship between the phenomena of signal, sense, and sentience in plant life.

The paper explores the possibility that anthropomorphism is more and other than what we long thought it to be. Rather than a one-way imposition of human metaphors onto plants, this paper explores the concept of “phytomorphism” to examine ways that plant researchers *vegetalize their sensorium* in the process of reaching toward deeper understandings of plant life.

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## Poster abstracts

# THE ROLE OF HYDROGEN PEROXIDE IN PROPAGATION OF VARIATION POTENTIAL IN *PISUM SATIVUM* L.

**Akinchits E, Katicheva L, Sukhov V,  
Semina M, Bushueva A and Vodeneev V**

Variation potential (VP) in higher plants is induced by external damage. VP represents the local electric response to distribution of a hydraulic or chemical signal. Still the question of the nature of the signal inducing VP remains controversial. Hydrogen peroxide can quickly spread on a plant and induce development of electric reaction. In the present work the analysis of a role of  $H_2O_2$  as inductor VP is carried out. Experiments were performed on two-week seedlings of pea (*Pisum sativum* L.). VP was induced by a leaf tip burning. Electrical activity was recorded extracellularly. The maintenance of  $H_2O_2$  was recorded by fluorescent probe Oxi-Red. The plant was fixed in liquid nitrogen in different time intervals after burning. There were allocated three fragments of the stem. Fragments homogenized in TCAA, with the subsequent evaluation of  $H_2O_2$  concentration in homogenate. Burning caused VP distribution in stem with velocity equaling to  $1\div 4 \text{ mm s}^{-1}$ .  $H_2O_2$  concentration was not changed in 25 s after burning. In 50 s after that  $H_2O_2$  concentration rise was registered in the first fragment and in 100 s it returned to the initial level. In the second fragment the content of peroxide grew in 100 s after burning and came back to initial level in 200 s. In the third fragment tendency to  $H_2O_2$  concentration rise in range from 50 to 200 s after the burning was observed. In a separate series of experiments with intracellular electrical activity registration it was shown that the increase of the  $H_2O_2$  concentration in the washing solution causes depolarization development.

Amplitude of this depolarization was comparable to that for VP. The obtained results specify that  $H_2O_2$  may be considered as the agent inducing VP.

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# PHENOLIC COMPOUNDS AND INCIDENCE OF *MONILIOPTHORA RORERI* IN COCOA FRUITS

**Albores-Flores V, Adriano-Anaya ML, Gutierrez E, Ovando I, Salvador-Figueroa M**

In Mexico, frosty pod rot (*Moniliophthora roreri*) of cocoa fruit is a highly destructive disease. The loss of fruit can be up to 100%. It is known that in different plants, hemi-bio-trophic fungal attack induces different metabolic pathways and, as a consequence, the production and accumulation of metabolites of defense (i.e. lignin, hydrolytic enzymes and phenolic compounds). In this work, we studied the effect of two plant extracts (*Allium sativum* and *Syzygium aromaticum*) and *Streptomyces* spp EPCH0496 (biomass and fermented medium) in the content of total phenolic compounds and the incidence of frosty pod rot in fruits of cocoa Trinitario variety. In a plantation of cacao trees (14° 59' 51"N; 92° 10' 51" W; 460 masl), eleven treatments (1 ha per treatment and 70 trees ha<sup>-1</sup>) were established. Every 14 days, extracts and bacteria, alone or in combination, were applied on the surface of the fruits. The fruits (40 per treatment) were harvested after 14 weeks of growth. Frosty pod rot incidence and phenolic compounds (PC) content of the pericarp was quantified. The fruits of treatment with *A. sativum* + *S. aromaticum* + *Streptomyces* spp biomass had the highest amount of PC (3.853±0.175 meq gallic acid g<sup>-1</sup>) and lower incidence of disease (30%). The PC content and the incidence of disease in the fruits of control treatment were 2.103±0.145 meq and 78%, respectively. In the remaining nine treatments the PC content and the incidence of disease ranged from 2.663 to 3.326 meq and 51 to 70%, respectively. An inverse relationship between PC content and incidence of disease was found.

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# MELATONIN SYSTEMICALLY AMELIORATES DROUGHT STRESS-INDUCED DAMAGE IN *MEDICAGO SATIVA* PLANTS

**Antoniou C, Chatzimichail G, Xenofontos R, Pavlou G, Panagiotou E, Karmiotis K and Fotopoulos V**

Terrestrial plants are constantly exposed to multiple abiotic stress factors such as drought, salinity and heat. Melatonin (Mel; *N*-acetyl-5-methoxytryptamine) is a naturally occurring metabolite, which is involved in multiple physiological processes in plants. The present study attempts to investigate the effect of this molecule in drought-stressed *Medicago sativa* L. plants. Plants were initially pre-treated with Mel by soil watering and were then subsequently exposed to severe water deficit. Preliminary experiments examined the extent of cellular damage in leaves by determining lipid peroxidation (MDA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and nitric oxide (NO) content. Interestingly, drought-stressed plants pre-treated with Mel demonstrated significantly lower cellular damage levels compared with non-primed stressed plants, while the primed plants also showed lower reactive oxygen and nitrogen species content. In addition, primed and subsequently stressed plants displayed improved physiological performance in terms of increased chlorophyll fluorescence (indicative of photochemical efficiency of PSII) compared with non-primed, stressed plants. Real-time RT-PCR analysis and enzymatic activity assays are currently underway, examining the expression and activity levels of key defense-related antioxidant enzymes. Our results propose an important role for Mel as a systemic plant priming agent against drought stress conditions, while further experiments are planned which will attempt to delve deeper in the *modus operandi* of this compound.

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# IMPACT OF THE OLIVE TREE PHENOLIC COMPOUND OLEUROPEIN ON *PECTOBACTERIUM* INDUCED CELL DEATH IN TOBACCO CULTURE CELLS

**Arbelet-Bonnin D, Biligui B, Bouteau F**

More than 8 million ha of olive trees (*Olea europaea* L.) are cultivated worldwide. Olive tree pruning is a largely available ligno-cellulosic residue especially in the Mediterranean countries accounting for almost 98% of the world crop. It has been estimated that an average of 3 tons of pruning biomass is obtained every year from one olive tree hectare. It makes these residues a huge, cheap, and unexploited energy or chemicals source. Oleuropein is the major phenolic compound among the phenolic compounds identified from olive wood. Oleuropein was shown to exhibit several biological properties, many of which may result from their antioxidant and free radical scavenger activity. It was evaluated for its *in vitro* antimicrobial activity.

We recently showed that plant cultured cells could be an alternative tool to evaluate rapidly and efficiently the virulence of different *Pectobacterium* spp. strains, economically important plant pathogens that cause plant soft rot, (Terta et al. 2010). Such plant cell cultures are also useful to record the efficiency of pretreatment by molecule-derived from biocontrol agents (Baz et al. 2012).

Here we showed, using tobacco BY2 cell cultures, that pretreatment with oleuropein could reduce the virulence of *Pectobacterium atrosepticum*. This effect could be due to its antioxidant activity since oleuropein also decrease the extent of reactive oxygen species generation-dependent cell death induced by the lipopolysaccharide (LPS) of *Pectobacterium* (Kettani-Halabi et al. 2015).

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# NEW *ARABIDOPSIS* MUTANTS WITH A REDUCED PRIMARY ROOT GROWTH RESPONSE TO LOW-PHOSPHATE

**Balzerque C<sup>1</sup>, Laugier E<sup>1</sup>, Godon C<sup>2</sup>, Teulon J-M<sup>2</sup>, Brouchoud C<sup>1</sup>, Dartevelle T<sup>1</sup>, Bissler M<sup>1</sup>, Creff A<sup>1</sup>, Delannoy E<sup>1</sup>, Thibaud M-C<sup>1</sup>, Pellequer J-L<sup>2</sup>, Nussaume L<sup>1</sup> and Desnos T<sup>1</sup>**

Phosphorus is an essential macro-element required for all organisms. The phosphate (Pi) starvation condition is a common situation for plants, which activate metabolic and developmental responses. We are interested by the *Arabidopsis thaliana* (Col-0) primary root growth inhibition by the low-Pi condition. We have shown that this inhibition depends of the root tip contact to the medium and we have identified the multicopper oxidase LPR1 as a major protein involved in this response. Here we present two new genes participating to this response: STOP1 and ALMT1. STOP1 is a transcription factor regulating the expression of ALMT1 and they are both already known for their role in the root resistance to low-pH and toxic aluminum, however their role in low-Pi was not yet documented. Kinetic analyses show that the WT root growth starts slowing shortly after seedling transfer on low-Pi, whereas the mutants behave like in high-Pi condition. We will present more details about the *stop1* and *almt1* mutants isolated, including Atomic Force Microscopy (AFM) nanomechanical analyses of the root tip surface after transfer of the plant on the low-Pi stress.

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# PHENOLIC COMPOSITION OF TUNISIAN ATRIPLEX SPECIES AND THEIR ANTIOXIDANT ACTIVITIES

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Alain B<sup>2</sup>, Riadh K<sup>1</sup>, Abdelly C<sup>1</sup>**

*Atriplex* plants are known by common name of saltbush because of their high tolerance to salt. In addition to being of great interest as fodder, some parts of *Atriplex* plants have even been used in human food. *Atriplex* plants are also known to be used in traditional medicine. Recent researches demonstrated therapeutic performances of these plants (antidiabetic and anti-herpes virus activities). These multiple medicines values are due to some chemical substances such as polyphenolic compounds known by their antioxidant action. The present study aims to assess antioxidant activities of methanolic extracts of three Tunisian *Atriplex* species (*A.halimus*, *A.inflata* and *A.nummularia*) harvested in March from their natural habitat. Various experimental models were used for characterization of antioxidant activities of leaf extracts. In addition, the total phenolic, flavonoid, and condensed tannin contents were determined. Results showed a significant difference in the antioxidant capacities of the studied species. The highest radical scavenging ability was found in *A.inflata*, followed by *A. halimus* and *A. nummularia*. *A.inflata* also exhibited the highest antioxidant ability against the inhibition of  $\beta$ -carotene bleaching, and a better total antioxidant capacity. Nevertheless, *A.halimus* showed the best reducing activity with the lowest IC<sub>50</sub> value. Moreover, *A. inflata* exhibited the highest phenolic levels. The identification of phenolic compounds in *A.inflata* leaves extracts using LC-MS revealed that quercetin glycosides and the feruloylquinic acid, well known for their high antioxidant activity, were the major molecules.

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# PRESENCE OF AN EXCLUSIVE CAPACITY IN THE SUCCULENT OBLIGATORY HALOPHYTE *CAKILE MARITIMA*

**Ben Hamed I<sup>1,2</sup>, Arbelet-Bonnin D<sup>1</sup>, Biligui B<sup>1</sup>, Ben Hamed K<sup>2</sup>, Abdely C<sup>2</sup>, Bouteau F<sup>1</sup>**

Salinity is one of the major abiotic stress factors that drastically affect plants growth, development and productivity. Halophytes growing in highly saline soils could serve as a resource for the identification and development of new crop systems for marginal saline soils. *Cakile maritima*, the sea rocket, is an annual succulent halophyte frequently found from the Black Sea coasts to the Mediterranean region and from the Atlantic coasts of North Africa to the north of Europe. Tunisian accessions of *C. maritima* contain up to 40% seed-oil. Plant growth and seeds produced per fruit segment is maximal at 100 mM NaCl. *Cakile maritima* is considered as an obligatory halophyte. It is known as a Na<sup>+</sup>-includer which accumulates Na<sup>+</sup> in aerial part and proline and sugars for osmotic adjustment.

Using suspension cells of *C. maritima* we showed that it could also present an excluder behavior. Using the Na<sup>+</sup> sensitive probe CoroNagreen we recorded a dose dependent increase in intracellular Na<sup>+</sup> which could be decreased by inhibitors of non selective cation channels (NSCCs) known to allow Na<sup>+</sup> influx. This influx was effectively correlated to a cell depolarization and an increase in NSCC currents. However, we also observed a rapid decrease in fluorescence due to Na<sup>+</sup> efflux from the cells. This efflux is correlated with the cell repolarization. Na<sup>+</sup> efflux from plant cells is known to be driven by the SOS system involving a H<sup>+</sup>/Na<sup>+</sup> antiport energized by plasma membrane H<sup>+</sup>-ATPases.

We effectively observed an acidification of the external pH upon addition of Na<sup>+</sup>. Furthermore H<sup>+</sup>-ATPase inhibitors allowed reducing the Na<sup>+</sup> efflux observed after a few minutes, strongly suggesting the involvement of a "SOS like" system in *C. maritima*.

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# **DIVERSITY AND DYNAMICS OF THE VEGETATION OF THE WESTERN ALGERIA**

**Bouazza M, Babal I**

The Algerian tells, and more specifically the region of Tlemcen, presents a flora and vegetation of the most remarkable of Algeria in their diversity.

We are currently witnessing a use of this wealth by humans for purposes of survival with an acceleration of the use of these resources beyond their capacity for renewal. In terms of vegetation dynamics, the western part of the Algeria match invasion of sclerophyllous structures alaternus (Rhamnus), pistachio (Pistacia), Juniper (Juniperus) etc., that are resistant to environmental stress but are highly susceptible to fire. The means process is initiated; it remains tied to climate variables and pressure anthropozoogenic; the clearest consequence is the extension of the desert vegetation areas.

KEY words: vegetation; Dynamics; Pistacia; Juniperus; invasion; Tlemcen; Algeria.

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# INVOLVEMENT OF HYDROGEN SULFIDE IN GERMINATION AND DORMANCY RELEASE OF ARABIDOPSIS SEEDS.

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**Puyaubert J and Bailly C**

Seed germination is a critical step of plant development and is strongly influenced by environmental parameters such as temperature, humidity or light. To optimize the chance of successful seedling development after germination, seeds are frequently dormant at maturity and will only acquire germination ability after dormancy release. As a variety of exogenous (temperature, light...) and endogenous signals (ethylene, gibberellins...) can trigger dormancy release, the underlying signaling networks and their interconnections are actively investigated.

Hydrogen sulfide recently emerged as a new intracellular messenger implied in plant responses to abiotic stress and plant development. Nevertheless, its participation and possible functions during plant seed germination has been poorly addressed.

We used dormant and non-dormant Arabidopsis seeds as a model for germination and dormancy release. Treatments with the H<sub>2</sub>S donor sodium hydrosulfide (NaHS) slowed down the germination of non-dormant seeds in a dose-dependent manner. A similar effect was observed on sunflower seeds. On the other hand similar NaHS treatments failed to release seed dormancy. Interestingly treatments with two inhibitors of H<sub>2</sub>S formation, hypotaurine and propargylglycine, also delayed seed germination. The activity of different cytosolic enzymes responsible for H<sub>2</sub>S synthesis (L- and D-cysteine desulfhydrases and -cyanoalanine synthase) increased within the first 24h of imbibition. In good agreement H<sub>2</sub>S content significantly increased after 24h of imbibition.

The germinative capacity of wild-type and *des1* (which is deficient for the cytosolic L-cysteine desulfhydrase) seeds was compared. No significant differences were observed between WT and *des1* seeds in the different conditions tested, suggesting that the activity of this enzyme is not necessary for proper germination, although it varies during this process.

Together our data evidenced a regulatory function for H<sub>2</sub>S during seed germination associated with an increase of H<sub>2</sub>S synthesis. The identification of H<sub>2</sub>S source(s) and targets should shed light on H<sub>2</sub>S signaling role in seeds

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# DYNAMICS OF THE FIVE CK RECEPTORS IN APPLE TREE CHALLENGED WITH PATHOGENS

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In plants, cytokinins (CK) regulate numerous developmental and physiological processes and their implication in plant-pathogen interactions has been recently unveiled. The characterization of CK signaling is a prerequisite to understand CK role in response to biotic stress. We aim at understanding the CK receptors dynamics in apple tree *Malus x domestica* challenged with pathogens through mechanistic and activity approaches. By *in vivo* imaging in plant cells and yeasts, we determined the subcellular localization and homodimerization of the five CK receptors MdCHKs and we showed that CK signal application influences their spatial dynamic behavior. Using yeast complementation studies, we developed a bioassay system to test the specificity and sensibility of MdCHKs towards various CK structures, allowing us to underly distinct properties for each receptor. Taking advantage of our yeast bioassay, we established that *Erwinia amylovora*, the bacterial pathogen responsible for the fire blight disease, activates some of the MdCHKs suggesting their probable role in this plant-pathogen interaction.

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# RELATIONSHIP BETWEEN GRAZING INTENSITY AND QUALITATIVE AND QUANTITATIVE CHANGES IN ARTEMISIA SIEBERI BESSER ESSENTIAL OIL COMPOUNDS IN KASHAN PROVINCE OF IRAN

**Dehghani Bidgoli R<sup>1</sup>, Marzieh YN<sup>2</sup>**

The relationship between grazing intensity and qualitative and quantitative changes in *Artemisia sieberi* Besser essential oil compounds in Kashan province of Iran was evaluated in this study. For this purpose, vegetation sites of *Artemisia sieberi* under three grazing intensities (heavy, moderate, and ungrazed) with the same ecological conditions based on the distance to the water resource were selected in Kashan-Ghamsar road rangelands and near Natural Essential Oils Institute, University of Kashan, Iran. Within each site, individual plants were sampled at random in the full flowering stage in a completely randomized (CR) design with three replications. The oils were extracted by hydrodistillation of the air-dried samples and were analyzed by GC-MS (Gas Chromatography–Mass Spectrometry). The main constituents of the oils were as follows: ungrazed site; 1,8-cineol (29.9%), myrcene (14.1%), moderate grazed site; myrcene (15.9%), 1,8-cineol (15.1%), Eudesm-7(11)-en-4-ol (11.1%), and heavy grazed site; 4-terpenyl acetate (23.3%), davanone (21.9%), p-cymene (19%). Among the compounds from the three sites, 4-terpenyl acetate allocated the highest amount in heavy grazed site. The analysis of essential oils showed that there were significant quantitative and qualitative differences found within the natural populations under the three different grazing sites.

Keywords: *Artemisia sieberi*, grazed, ungrazed, grazing intensities, essential oils.

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# **B-TYPE RESPONSE REGULATOR RR18 SILENCING TO DETERMINE ITS IMPACT ON POPLAR RESPONSES TO DROUGHT**

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F Brignolas<sup>1,2</sup>, F Héricourt<sup>1,2</sup>, D Auguin<sup>1,2</sup>  
A Oudin<sup>4</sup> and S Carpin<sup>1,2\*</sup>**

In the context of global climate warming, soil water availability will clearly impact plant growth. Their responses face to this hydric constraint will depend on stress perception. In *Arabidopsis*, some proteins have been identified as clearly involved in an osmosensing signaling pathway. These proteins belong to a system called multistep phosphorelay composed by three partners: histidine-aspartate kinase (HK) receptors, histidine phosphotransfer (HPT) proteins and response regulators (RR), including B-type RR (RR-B) which are transcription factors. In *Arabidopsis*, the receptor AHK1 has been identified as an osmosensor involved in drought perception. Among the five *Arabidopsis* HPTs, AHP2 appears to interact with AHK1. Furthermore, recent studies showed that ARR18 is involved in *Arabidopsis* osmotic stress responses. In poplar, HK1, the homologous protein of AHK1, was isolated and among the ten HPTs isolated, three of them interact with this receptor. Based on their interaction with these three HPTs, six of the nine isolated RR-B, including RR18, could participate to the osmosensing signaling pathway.

The *RR18* expression regulated by osmotic stress in different poplar organs led us to further study the RR18 function and to determine its involvement in the osmosensing signaling pathway by switching off the corresponding gene by a RNAi strategy. First, an *in silico* analysis was achieved to

determine a RR18 specific sequence for the hairpin construction. The corresponding sequence was isolated from *Populus tremula* x *P. alba* 717-1B4 genotype, commonly used for *Agrobacterium* transformation. The *RR18* insert was then cloned before sequencing. After triparental mating technique, the transformed *Agrobacterium* strain carrying the RNAi construction was used for 717-1B4 transformation. We obtained transformed *calli* and the analysis of this material is still in progress.

This work should help grasping details on the involvement of this RR in the poplar osmosensing signaling pathway.

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# **ALTERED SEED GERMINATION 2 (ASG2) IS A NOVEL FARNESYLATED DWD PROTEIN IDENTIFIED IN *ARABIDOPSIS THALIANA***

**Dutilleul C, Blanc N, Ribeiro I, 'Nezames C, Courtois M, Labas V, Giglioli-Guivarc'h N and Ducos E**

Protein isoprenylation is a post-translational lipidation process that facilitates protein membrane anchorings or protein-protein interactions. It consists in the addition of a prenyl moiety on the cysteine of a specific C-terminal CaaX motif of some proteins. It is in part realized by the farnesyltransferase (PFT) and the geranylgeranyltransferase type I that recognize the CaaX motif. The role of protein isoprenylation in plant has been notably highlighted by the characterization of the PFT Arabidopsis KO mutant, *era1* (Enhanced Response to ABA 1). *era1* plants show pleiotropic phenotypes including delayed growth and an increased sensitivity to ABA in seed germination and stomatal movement. Until now, the farnesylated CaaX proteins responsible for these phenotypes remain unknown. The identification and characterization of isoprenylated proteins is thus an important challenge to decipher the significance of isoprenylation in plant. In the present work, we adapted for Arabidopsis cell suspensions, a method developed for mammal cells that allows the *in vivo* tagging and capture of farnesylated proteins. Mass spectrometry analyses identified, among others, ASG2 (ALTERED SEED GERMINATION 2), a protein previously associated to the seed germination network. ASG2 is a conserved protein in plants and displays a unique feature that associates WD40 domains and tetratricopeptide repeats.

Additionally, ASG2 has a C-terminal CaaX motif that is farnesylated *in vitro*. *In vivo*, defect in ASG2 farnesylation provokes its exclusion from the nucleus, and affects its interaction with DDB1 (DAMAGE DNA BINDING protein 1), a protein of the E3 ubiquitin ligase complex. Finally, experiments performed on *asg2* and *era1* mutants reveal that they behave in a similar way when exposed to ABA or salt stress. To our knowledge, ASG2 is the first farnesylated DWD (DDB1 binding WD40) protein related to ABA responses in Arabidopsis that may be linked to *era1* phenotypes.

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# GROWTH RATE IN A MECHANICALLY STRESSED ENVIRONMENT: SIZE MATTERS !

**Fadlallah H, Jarrahi M, Herbert E, Peerhossaini H**

Biofuels offers a transition towards a renewable world of energy supply and production. Third generation of biofuels produced from non-feed stocks such as microalgae and cyanobacteria, potentially offers great opportunities that have greatly increased recently, since they have higher CO<sub>2</sub> fixation rates than plants. However, cost effective production in large scales remains a major challenge to overcome. Thus, optimizing a growth system with highest biomass productivity is a necessity.

Effects of mechanical forces and shear stress on the growth rate of cyanobacteria *Synechosysits PCC 6803* and microalgae *Chlamydomonas* cells are studied and investigated in a mechanically agitated vessels. Thus, an experimental setup was prepared to quantitatively monitor the growth rate versus the shear rate.

3 multi-slot digitally controlled magnetic agitators are placed inside a closed chamber provided with air flow through a 12 V ventilator used to maintain air circulation inside the chamber, under a light intensity of 450 Lux ( $6 \mu\text{E m}^{-2} \text{s}^{-1}$ ), a 6 watts white fluorescent light source (2700 °k) was used to provide the light, experiment was done under full time light periods over 4 weeks.

The experiment was done inside a clean room, by which the temperature was adjusted to 20 ° C, at atmospheric pressure.

In order to study the effect of stress variation on the growth rate, different frequencies of agitation are tested (3, 6, 9 hz), 2 vessels filled with 150 ml of each specie is placed on each agitator, and sealed with a cotton from the top.

The growth is monitored daily using a UV/Vis spectrophotometer to measure the optical density versus wavelength and time and thus correlate to the cellular concentration using a microscopic cell counter, the PH is adjusted to 7 all over the experiment in order to maintain the photosynthetic activity of the species.

Results show that the growth rate is independent of the shear stress magnitude, mostly for *synechosysits*, and with a lower rate for *chlamydomonas*, depending on the cell size of each specie. Furthermore, and in order to have accurate approach on the shear stress distribution and its magnitude, a special non-destructive method of measurement based on particle image velocimetry "PIV" was done, in order to study precisely the velocity field inside our system, and thus calculate the shear stress, and specify the zones with high shear from the ones with low one, as well as calculating other parameters that could be useful for our study and analysis (vorticity, deformation, distribution of velocity vectors...).

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# WHOLE TRANSCRIPTOME SEQUENCING AND ECOPHYSIOLOGICAL TRAITS TO QUALIFY DROUGHT RESPONSES IN *POPULUS NIGRA* GENOTYPES

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Climate evolution will tend to an increase of frequency and severity of droughts. These changes may alter the distribution and potential survival of plant species. Drought treatment observed on trees induces diverse responses in function of ecological strategies which depend on genotype x environment interaction. Earlier modifications of ecophysiological traits (stomata closure, photosynthesis decrease, and hydraulic tension increase) in response to drought stress can be qualified as isohydric as opposed to anisohydric behaviour. Then our objective was to test if these contrasted phenotypes had a specific transcriptional regulation. Production and hydraulic traits were monitored during severe stress on six *Populus nigra* genotypes in order to bring out ecophysiological traits which are genotype-specific and to characterise drought-response strategies.

Multivariate analysis on ecophysiological traits allowed us to choose the most indicative ones of hydraulic and physiological plant status and to define iso- or anisohydric genotypes. Total RNA sequencing was performed by RNAseq, on new leaves formed during stress, sampled at the maximum drought stress intensity indicated by predawn leaf water potential. A statistical analysis of transcripts expression levels was carried out to clean out the data and to reveal trends. Afterwards, these informations were analysed along with genes groups involved in leaf blade water regulation to estimate relationships between expression level and ecophysiological responses.

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# MECHANO-SENSING IN MEDICAGO ROOT HAIR DURING LEGUME/SYMBIONT INTERACTION

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Root hair not only has a major role in water and nutrient uptake but it also plays a central role in the establishment of nitrogen-fixing symbiosis in Legume plant. In our approach we took advantage of the knowledge on the genome and on the transport system in Arabidopsis to address an issue specific to Legume. In this study we specifically address the question of the role of mechanosensitive channels in early interaction between the model legume *Medicago truncatula* and Sinorhizobium.

During the early symbiotic interaction between Medicago and its microsymbiont, the infection is initiated by bacterial adhesion to root hairs and will lead to hair curling entrapping the bacteria. The hypothesis of the involvement of physical sensors during these early events is worth considering. In plant, the molecular nature of protein mediating mechanoperception is still elusive. Mechanosensitive ion channels belonging to MscSL (Mechanosensitive Small-conductance Like) family represent suitable candidates to be involved in this process. MscSL proteins have been recently characterized in Arabidopsis in our laboratory. *In silico* analyses of Medicago's genome lead us to identified six homologs putatively addressed at the plasma membrane. Two of them are present in root hair. Preliminary results showed that one of these provides a channel activity dependant on membrane tension. Functional characterization of the role of these root hair channels in early symbiosis is in progress.

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# OVEREXPRESSION OF ROOT SPECIFIC RICE PR10 (RSOSPR10) CONFERS TOLERANCE AGAINST DROUGHT AND SALT IN RICE AND GRASS PLANTS

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RSOsPR10 was originally found as a root specific protein induced by drought and salt treatment in rice. The mRNA and protein were induced specifically in roots by drought, and salt, and but not by low temperature and abscisic acid (ABA). In our series of studies, it was revealed that the transcription is up-regulated by jasmonic acid (JA), while salicylic acid (SA) strongly inhibits this induction (Takeuchi *et al.*, Plant Cell Physiol, 2011). Immunohistochemical and *RSOsPR10 pro::GUS* reporter experiments showed the expression of RSOsPR10 in the cortex cells of roots and especially at lateral root primordia. To know the physiological function of RSOsPR10 in plants, we regenerated RSOsPR10 overexpression (OX) lines of rice and bentgrass. RSOsPR10 OX rice showed highly tolerance against drought stress, and RSOsPR10 OX bentgrass conferred both drought and salt tolerance. There was almost no difference in phenotype and growth of aerial parts in the OX plants, comparing to non-transgenic plants. However, their root growth and total masses were significantly higher in the transgenic plants. It is thus postulated that RSOsPR10 involves the tolerance against environmental stresses, via increasing root development and growth. We are in progress the growth test of RSOsPR10 OX rice under biotic and abiotic stresses in the field condition.

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# MOLECULAR STUDY OF A SECOND HISTIDINE- ASPARTATE KINASE PUTATIVELY INVOLVED IN OSMOSENSING SIGNALING PATHWAY IN *POPULUS*

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The osmosensing pathway in *Arabidopsis thaliana* is constituted by a multi-step phosphorelay similar to the one of *Saccharomyces cerevisiae*, involving a Histidine-aspartate Kinase (HK) osmosensor, AHK1, Histidine-containing Phosphotransfer proteins (AHPs) and Response Regulator (RRs). In *Populus*, we have isolated a cDNA encoding a HK, named HK1, ten cDNAs encoding HPT proteins, HPT1 to HPT10 and nine cDNAs encoding RR proteins. Different pieces of evidence prompted us to suggest that HK1 could act as an osmosensor in *Populus*. This protein shares 83.65 % of similarity with AHK1, is localized to plasma membrane *in planta*, is able to homodimerize in yeast and *in planta* and to complement an osmosensing-deficient yeast in osmotic stress conditions. An interaction analysis, in yeast and *in planta*, showed that HK1 can interact with different HPTs, among which HPT2, HPT7 and HPT9 being preferential partners.

Recently, we identify another gene presenting homology to HK1 in the genome of *Populus*, constituting a paralog of HK1 and named HK1b (HK1 was renamed HK1a as a consequence). This paralogous protein shares 96.35 % of similarity with HK1a and has been study in order to understand its link with the previously characterized HK1a protein. Therefore, we showed that HK1b is able to form heterodimers with HK1a and can complement an osmosensing-deficient yeast in osmotic stress conditions in a better

way than HK1a. Moreover, HK1b can interact with different HPTs in a similar way compare to HK1a. These results raise the question about the role of this paralogous protein in the osmosensing signaling pathway in *Populus*.

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# PHYSIOLOGICAL RESPONSES OF *HORDEUM MARITIMUM* TO SALINITY

**Hmidi D, Messedi D, Farhani F, Ben Daly A and Abdelly C**

The purpose of the present study is to compare the short time response to salt stress of two *Hordeum maritimum* accessions: Kalbia and Soliman. Plants were exposed to different salt concentrations (0, 100, 200, 300 and 400 mM NaCl) and harvested after 4h, 24h, 48h and 7 days of treatment. Measured parameters were the relative water content, photosynthetic parameters and chlorophyll fluorescence. The two *H. maritimum* Tunisian accessions (Soliman and Kalbia) appear to be tolerant to salt at higher concentrations; thus, tissue remains relatively hydrated even at 400 mM NaCl. Quantifying the photochemical efficiency of photosystem II (PSII) shows that  $F_v/F_m$  was not affected and maintained constant as a function of time and the salt concentration of the medium suggesting the absence of photoinhibition in both accessions. Salt enhanced the activity of PSII at 100 and 200 mM in Soliman (Quantum yield  $\Phi_{PSII}$  increased) suggesting a positive effect in the efficiency of PSII photochemistry. In Kalbia, the activity of PSII was enhanced at 100, 200 and 300 mM, however, this activity decreased at 400 mM NaCl in two accessions. This decrease was accompanied by a reduction of the proportion of PSII opened reaction centres (photochemical quenching qP increased) in concomitance with the increase of NPQ. Our results suggest that the decline in the efficiency of PSII is a negative feedback due to the decline of the photosynthetic assimilation resulting from stomatal closure ; a mechanism adopted by both accessions for better salt tolerance.

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# WHEN OSGUN4 TALKS, WHO'S LISTENING?

**Jiang M<sup>1</sup>, Li R.Q<sup>1</sup>, Huang JZ<sup>1</sup> and Shu QY<sup>1</sup>**

*GENOMES UNCOUPLED4* (GUN4) was first identified in *Arabidopsis thaliana*, it plays important roles in chlorophyll biosynthesis and plastid-to-nucleus retrograde signaling, but the underlying molecular and biochemical mechanisms are yet largely unknown. The rice *GUN4* ortholog, *OsGUN4* was identified by map-based cloning of a yellow-leaf-color mutant gene. Interestingly, we discovered that the mutant trait is controlled by a down-regulating epi-allele of *OsGUN4*, with a hypomethylated segment containing an antioxidant response element in its promoter region. We determined that expression of *OsGUN4* was responsive to light intensity and treatment of H<sub>2</sub>O<sub>2</sub>, and the epigenetic mutation abolished its response in the mutant. We further observed that the *OsGUN4* mutation also affected the expression of many nuclear genes. Based on the results, we would propose a preliminary hypothesis describing the possible functions and working model of *OsGUN4* in the plastid-to-nucleus signaling pathway.

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# IMPACTS OF EXTRACELLULAR PH ON ROOT GROWTH AND TROPISMS: POSSIBLE ROLES OF EXTRACELLULAR ATP RECEPTOR DORN1 IN *ARABIDOPSIS THALIANA*

**Kagenishi T<sup>1</sup>, Yokawa K<sup>1,2</sup>, Stacey G<sup>3</sup> and Baluška F<sup>1</sup>**

It is well known that extracellular pH affects root growth and tropisms. External pH, regulated by proton gradients between the internal and external space, alters many physiological processes. Plant roots possess robust intrinsic mechanisms to control and maintain appropriate pH values on the root surface. Extracellular ATP (eATP), which is secreted via exocytosis, acts as extracellular signal, which plays a variety of physiological roles. For instance, eATP inhibits root growth and gravitropism. However, the molecular mechanisms of eATP action in the plant cell wall are not understood. It was reported that secreted eATP can lower pH in animal retinal cells. The aim of the present study was to investigate the involvement of eATP and extracellular pH in roots. *Arabidopsis* WT and eATP specific receptor (DORN1) mutant lines were used. The results showed that root growth was inhibited in 5 day-old seedlings at acidic pH (4.5), the *dorn1-1* mutant roots showed no inhibition at this pH value. Interestingly, alkaline pH (8.0) also inhibited root elongation, both in WT and the *dorn1-1* mutant roots within 24 hours. The inhibition recovered within 48h after treatment in WT roots but not in the *dorn1-1* mutant roots. These results indicate that DORN1 eATP receptor plays an important role not only in eATP perception but also in the adaptation of roots to acidic or alkaline environments.

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# **NUCLEUS: HUB OF REDOX-SIGNALING IN SEED PHYSIOLOGY?**

**Kalembe E, Xia Q, Bouteau F, Bailly C, El Maarouf Bouteau H, Meimoun P**

Reactive oxygen species (ROS) have been shown to be toxic but also function as signaling molecules in a process called redox-signaling. Cellular signaling transduction pathways enable organisms to receive signals and respond in specific manner. In seeds, ROS are produced at different developmental stages including dormancy release and germination. Recent data suggests that the nucleus via its effectors and transducers can also generate its own signaling of ROS. This data led us to focus on the determination if ROS formation can be generated directly by the nuclei in seeds of sunflower. Enriched nuclei fraction isolated from embryonic axes during imbibition was tested for the ability to produce ROS as a response to exogenously applied of hydrogen peroxide. The nuclear ROS production has been characterized as a superoxide anion production, was enhanced by calcium pretreatment, reversed by GSH and inhibited by diphenyleioidonium – the inhibitor of flavoenzymes. After purification of membrane proteins from isolated nuclei, the protein responsible of the nuclear ROS production was isolated to allow its identification. Interestingly, the isolated nuclei from dormant and non-dormant seeds differed in ROS production capacity after  $H_2O_2$  treatment suggesting a major role of nuclei in the integration of redox-signaling.

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# AXIS GROWTH DURING SEED GERMINATION DEPENDS ON COORDINATED ACTION OF PLASMA MEMBRANE-LOCATED NADPH OXIDASE AND H<sup>+</sup>-ATPASE

**Kar RK, Majumder A, Singh KL**

NADPH oxidase (NOX), described as Rboh in plants, plays a pivotal role in plant growth and development, as revealed from most recent works. Being located on plasma membrane it transports electrons from NADPH on the cytosolic side to reduce oxygen on apoplastic side across membrane. Animal NOX has an associated proton channel activity to prevent depolarization of membrane by proton efflux. However, no such proton channel activity has been reported for Rboh so far, although H<sup>+</sup>-ATPase has been well reported to play important role in cell elongation growth through cell wall relaxation in plants. In *Vigna radiata* seed germination and seedling growth is regulated by NADPH oxidase through ROS mediated cell elongation. Side by side, H<sup>+</sup>-ATPase also contributes to cell wall relaxation, as its inhibitor, sodium orthovanadate (Van) affects germination as well as seedling growth. Besides, activity of H<sup>+</sup>-ATPase may be revealed from lowering of apoplastic pH during germination and axis growth. Also, treatment with CCCP (protonophore), that dissipates proton gradient, retarded germination and axis growth significantly. Experiments to explore any possible interaction of NOX (Rboh) and H<sup>+</sup>-ATPase revealed a possible positive feedback loop.

Thus treatment with Van and CCCP lowers superoxide accumulation and affects NADPH oxidase activity. On the other hand, ZnCl<sub>2</sub> (NOX inhibitor) and CuCl<sub>2</sub> (O<sub>2</sub><sup>-</sup> scavenger) diminishes H<sup>+</sup>-ATPase activity in the growing axes. Ca<sup>2+</sup> has been found to be associated with axis growth via activation of both NOX and H<sup>+</sup>-ATPase, since activity of these enzymes depends on influx of apoplastic Ca<sup>2+</sup> as revealed by treatments with Ca<sup>2+</sup> antagonists (La<sup>3+</sup>, Li<sup>+</sup> and EGTA). Ethylene acts upstream and is required for activation of both NOX and H<sup>+</sup>-ATPase during germination.

A model on coordinated action of PM-located NOX and H<sup>+</sup>-ATPase downstream of ethylene in early stage of axis growth during germination of *Vigna radiata* seeds has been proposed.

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# **NITELLOPSIS OBTUSA AS MODEL SYSTEM FOR ENVIRONMENTAL IMPACT INVESTIGATION: CURRENT AND VOLTAGE CLAMP APPROACHES**

**Kisnieriene V, Lapeikaite I, Sevriukova  
O, Daktariunas A, Ruksenas O**

The bioelectrical response of the characean cell is rapid and highly sensitive to various chemicals and physical parameters. The algae *Nitellopsis obtusa* provides an ideal model system for investigation of instantaneous extracellular effects on the generation of plant bioelectrical signals *in vivo*. K<sup>+</sup>-anesthesia method and conventional glass-microelectrode technique in current-clamp and voltage-clamp modes are used for the registration and analysis of electrical parameters. Sufficient signal amplification and high discretization frequency enable detail investigation of action potential (AP) parameters: excitation threshold, AP peak and duration, membrane potential at various voltages and dynamics of ion currents, allowing precise, high time resolution analysis of real-time processes. Combination of these approaches allows to observe rapid effect of light stimulation, metals, neurotransmitters and radiation on cell physiology. We summarize results of our investigations of *Nitellopsis obtusa* electrical responses to various compounds and stimuli discussing possible perspectives. The electrophysiology of plants may be a useful technique to improve our understanding of signalling pathways and physiological processes in plant cells during various environmental impacts and stresses.

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# THE STIMULATION OF ACTIVITIES OF PARAMAGNETIC CENTERS IN CEREAL GRAINS AS THE RESPONSE TO UV-TREATMENT

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The aim of the present work was to investigate the influence of the direct UV irradiation of whole cereal grains and their parts (seed coats, embryos and endosperms) on generation of stable organic radicals and changes in the amount and character of bonding of transition metal ions (Mn(II), Fe(III), Cu(II)). The grains of cereals (wheat, oat, barley) originated from plants with different tolerance to oxidative stress. Untreated whole grains and their parts exhibited EPR signals of stable organic radicals: semiquinone, tyrosyl and carbohydrate, as well as transition metal ions. The character and amounts of these species depended on the kind of cereal and on the part of grain. Seed coats exhibited the highest content of semiquinone, whereas tyrosyl radicals were present in both embryos and seed coats. In endosperms, the lowest EPR spectra intensity of carbohydrate radicals' species were found. Upon UV irradiation the amount of all radical species increased, especially in seed coats and embryos. The smallest changes of radicals amount observed for endosperms were, however, connected with formation of new paramagnetic species. The generation of radicals depended also on the kind of cereal and their tolerance to stress. The most sensitive to UV irradiation were oat grains, whereas grains of wheat and barley exhibited similar susceptibility to UV. In case of all cereals, the amount of radicals generated upon UV was higher in grains originating from sensitive genotypes.

Simultaneously, UV irradiation caused the increase of Fe(III) signal intensity in the spectra of seed coats, whereas the decrease of the intensity of Mn(II) signals in embryos was observed. The former resulted from oxidation of Fe(II), silent in EPR, to Fe(III). Vanishing of Mn(II) signals could be explained by destruction of Mn-protein complexes.

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# **ACTIN CYTOSKELETON IS NOT A TARGET IN UV-B SIGNALING IN *ARABIDOPSIS***

**Krzyszowiec W and Banas AK**

UV-B is a component of the sunlight spectrum. Its influence on cells is being widely studied at present. It was shown that microtubules in plant cells undergo reorientation under the influence of UV-B. Moreover, in animal cells UV-B causes actin cytoskeleton alterations. The aim of this study was to investigate actin architecture after UV-B irradiation in *Arabidopsis*. Samples were treated with 4 W/m<sup>2</sup> of UV-B. Two methods of actin visualization were employed. The actin cytoskeleton was either stained with Alexa Fluor 488 Phalloidin in fixed *Arabidopsis thaliana* (Columbia) leaves or visualized using the GFP-FABD2 construct in transgenic *Arabidopsis* plants. First we investigated the microfilaments after 10 and 60 min of UV-B irradiation to observe the immediate response to UV-B. Next we probed actin after 10 and 60 minutes of UV-B followed by 2 h of darkness. Only longer irradiation caused some damage to the actin cytoskeleton. However we noticed that the transgenic plants died after 2 days of culture after 60 min UV-B treatment. We suggest that the processes of necrosis or apoptosis are unrelated to alterations in the actin cytoskeleton.

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# **ACTION POTENTIALS ELICITED IN THE LIVERWORT *MARCHANTIA POLYMORPHA* BY ILLUMINATION AND DARKENING.**

**Kupisz K, Dziubińska H, Kwiecień E,  
Trębacz K**

Using the microelectrode method, bioelectrical changes in the membrane potential were studied after darkening and illuminating of the *Marchantia polymorpha* thallus. The average value of the resting potential in the cells of thalli was at the level of  $-166.0 \pm 4.2$  mV.

Turning off the light evoked generation of action potentials (APs) in *M. polymorpha* cells whose average amplitude value was  $90 \pm 11$  mV and the half-time was  $32 \pm 2$  s. The amplitude of APs did not depend on the time and intensity of the light preceding darkening. In part of plants darkening evoked action potential-like responses consisting of relatively fast depolarization, long-lasting plateau (in minutes range) and slow repolarization. The lag-time of APs evoked by darkening was in the range from 1 to 50 min. The illumination of the *M. polymorpha* thallus caused generation of APs as well. To evoke APs in response to illumination, application of 30-min darkening and a threshold value of light intensity of 120  $\mu\text{mol}/\text{m}^2\text{s}$  were required. The amplitude of APs evoked by illumination was  $103.4 \pm 5.4$  and the half time was  $59.0 \pm 7.1$  s.

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# **SLOW-VACUOLAR TYPE CHANNELS IN THE TONOPLAST OF THE LIVERWORT *MARCHANTIA POLYMORPHA*.**

**Kupisz K, Dziubińska H, Trębacz K**

The object of our patch-clamp studies was the liverwort *Marchantia polymorpha* – a plant phylogenetically located between algae and higher plants. In our experiments, the basic solutions were symmetrical in the bath and in the pipette and contained 100 mM KCl and 0.5 mM CaCl<sub>2</sub>. Patch-clamp measurements with whole vacuoles from *Marchantia polymorpha* resulted in slowly activating currents, typical for SV [slow-vacuolar] channels. These currents displayed a characteristic outward rectification i.e. cation flux from the cytoplasmic to the vacuolar side. The unitary conductance registered from isolated patches was  $67.8 \pm 1.2$  pS and  $27.3 \pm 2.5$  pS at 100 mV and  $34.5 \pm 1.3$  pS at -100 mV. A tenfold decrease in KCl in the bath shifted the reversal potential close to  $E_{K}$  and caused reduction of the SV unitary conductance at a value that was indiscernible in the noise. The gradient of KCl revealed the current at the negative potential with the unitary conductance of  $35.0 \pm 2.6$  pS. Registration of SV type channels was also obtained in experiments with the use of the solution with symmetrical composition of 100 mM Na-Gluconate in place of 100 mM KCl. However, such a replacement caused reduction of the unitary conductance SV channels to the value of  $32.0 \pm 0.4$  pS and diminished the negative currents.

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# PARAMAGNETIC SPECIES CREATED BY OZONE TREATMENT IN CEREAL GRAINS

**Łabanowska M<sup>1</sup>, Kurdziel M<sup>1</sup> and Filek  
M<sup>2</sup>**

The direct short-term ozone-treatment of the grains of three cereals (wheat, oat and barley), each represented by two genotypes with different oxidative stress tolerance was studied by electron paramagnetic resonance (EPR) technique. Investigations were performed on whole grains as well as on their parts: embryo, endosperm and seed coat. EPR method revealed in investigated plant materials the presence of transition metal ions (Fe(III), Cu(II), Mn(III)) and stable radicals, localized on organic molecules. It was found, that ozone application affected the chemical character and increased the amount of paramagnetic species, especially in sensitive genotypes. There were differences in the response to this stress-factor between studied cereals and between particular parts of grains. Embryos and endosperms appeared to be more susceptible to ozone in comparison to seed coats. In endosperms, ozone treatment increased mostly the number of carbon centered radicals located in carbohydrate structures, whereas in embryos also the increase of tyrosyl radicals occurred. In seed coats, besides the latter, the rise in EPR signal intensity of semiquinone radicals was observed. The greatest differentiating between the genotypes of various tolerance to stress was found for endosperm where contributions of particular carbohydrate radical signals depended on the oxidative stress susceptibility of studied cultivars.

Moreover, the increase of the intensity of tyrosyl radical discriminated the genotypes with different stress tolerance, being higher in sensitive ones. On the other hand, the content of radicals in seed coats was correlated with plant stress tolerance only in the small degree. Contrary, the increase of signal intensity of transition metal ions was the most visible in spectra of seed coats, mainly for sensitive cultivars of all grains.

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# MODELLING OF NITROGEN UPTAKE BASED ON A CROSS-COMBINATION OF *FLOW-FORCE* INTERPRETATION OF NITRATE UPTAKE ISOTHERMS AND ENVIRONMENTAL AND *IN PLANTA* REGULATION OF NITRATE INFLUX

**Le Deunff E<sup>1</sup>, Malagoli P<sup>2</sup>**

A mechanistic structural–functional model was developed to predict nitrogen uptake throughout the growth cycle by a crop of winter oilseed rape (*Brassic napus*) grown under field conditions. The functional component of the model (nitrate uptake activity) derives from a revisited conceptual framework that combines the thermodynamic *Flow–Force* interpretation of nitrate uptake isotherms and environmental and *in planta* effects on nitrate influx. The *Flow–Force* theory was proposed in the seventies to describe ion isotherms based upon biophysical “flows and forces” relationships of non-equilibrium thermodynamics. This perspective appears more realistic for describing root N uptake at kinetic and molecular levels than the carrier viewpoint of *Enzyme–Substrate* interpretation. Indeed, the *Enzyme–substrate* interpretation has not withstood recent molecular analyses about nitrate transporters. At least four families of nitrate transporters operating at both high and/or low external nitrate concentrations and located in series and/or parallel in the different cellular layers of the mature root are involved in nitrate uptake. Likewise, the demonstration was made that  $V_{max}$  and  $K_m$  parameters deduced from influx isotherms are only “apparent” parameters representative of the overall behaviour of the root sample studied for the absorption process.

Estimation of structural component of the model (root biomass) is based upon a combination of root mapping along the soil depth profile in the field and a relationship between the specific root length and external nitrate concentrations. The root biomass contributing actively to N uptake was determined by introduction of an integrated root system age that allows assignment of a root absorption capacity at a specific age of the root.

This conceptual framework provides a model of nitrate uptake that is able to respond in a more realistic manner to external nitrate fluctuations and to changes of climatic and *in planta* factors throughout the plant growth cycle at both functional and structural levels.

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# EFFECT OF COMMERCIALY AVAILABLE PLANT DEFENCE STIMULATORS ON HUMAN INNATE IMMUNITY

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PDS (Plant defence stimulators) constitute a recent alternative to pesticides used for crop protection. These compounds are of diverse nature, they all act by stimulating plant innate immune system and plants can better fight pathogens. There are many similarities in pathogen perception systems and cellular signalling in plants and animals. Many elicitors stimulate both human and plant innate immunity [Zipfel and Felix, 2005]. Therefore, it is likely that human innate immunity could be modulated by PDS. The aim of this study is to evaluate pro/anti-inflammatory activity of five different commercially available PDS on human cell models.

We studied the pro/anti-inflammatory effect of PDS on human peripheral blood mononuclear cells (PBMC). These cells are exposed during 20 h to various concentrations of PDS or their corresponding active molecules. Pro-inflammatory action is evaluated by measuring the quantity of the inflammatory cytokine IL-1 $\beta$  in the cells supernatants using ELISA test. To study anti-inflammatory effect, PBMC were treated with LPS to trigger a basal inflammatory response. We then checked if PDS delivered at the same time as LPS modified IL-1 $\beta$  production. In addition, in all the experiments, the viability is evaluated with a XTT test.

PDS, which were however used at equal or lower concentrations than in the fields, show different profiles in terms of cytotoxicity and inflammatory modulation. Our results indicate that PDS can differently interact with human innate immunity.

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# IS SEROTONIN A SIGNALING MOLECULE IN PLANTS?

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Serotonin (5-hydroxytryptamine) is a compound found in both animals and plants. Its role in animals is well documented, where it is known to function as a neurotransmitter and to be involved in regulation of sleep and mood. In contrast, little is known about its function in plants, particularly its role as a signaling molecule. The rice gene *CYP71A1* encodes a cytochrome P450 monooxygenase known as tryptamine 5-hydroxylase (T5H), which converts tryptamine to serotonin. Knockout mutations of *CYP71A1* result in a trait known as leaf lesion mimic in rice. However, quantitative-PCR and immunohistochemical analysis indicate that *CYP71A1* is mainly expressed in root tissues, and not in above-ground organs such as leaves. Comparative root proteome studies on the *CYP71A1* knockout mutant Jiazhe DB and its wild type parent Jaizhe B were carried out to elucidate the role of serotonin in plants using DIGE (2-D fluorescence difference gel electrophoresis). Proteins potentially involved in the phosphatidyl inositol double signaling pathway, e.g. the phosphatidylinositol (PI) transfer protein (1.93-fold less expression) and phosphoinositide phospholipase C (2.12-fold less expression), were shown to be differentially expressed. Furthermore, several Ca<sup>2+</sup> ion store and sensor proteins, such as the EF-hand domain containing protein (3.57-fold greater expression) and the calreticulin protein (2.53-fold greater expression), which are located downstream of the phosphatidyl inositol double signaling pathway, were also shown to be differentially expressed. These data suggest that serotonin might serve as a signaling molecule in the phosphatidyl inositol double signaling pathway in plants.

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# AUXIN-BINDING PROTEINS ABP1 AND ABP4 ARE INVOLVED IN EXPRESSION OF *PIN1A* IN LIGHT-DEPENDENT MANNER

**Malichová J, Plotzová R, Fellner M**

We previously reported that in maize, auxin-binding proteins (ABPs) are likely involved in light signaling. In *Arabidopsis*, it was revealed that PINs, proteins engaged in polar auxin transport, cycle between the endosomes and plasma membrane, and their distribution in the cell could be regulated by auxin and ABP1. We investigated whether maize ABP1 and ABP4 influence, in interaction with light, the expression of *PIN1a* gene. We applied a genetic approach consisting of analysis of 'loss-of-function' mutants in *ABP1* and *ABP4* genes. Our experiments showed that ABP1 and/or ABP4 play a role in maize seedling growth and development, and that their effects and functional relationship are organ- and light-dependent. For example, the data suggest that ABP1 and ABP4 participate in the maintaining of the sensitivity to blue light-induced inhibition of mesocotyl elongation. Expression data showed that mutations in *ABP1* and/or *ABP4* gene alter level of *PIN1a* transcript, while the results suggest that functional ABP4 is needed for *PIN1a* expression in the etiolated mesocotyls. Interestingly, mutant *abp1* showed high expression of *PIN1a* gene in blue light, whereas in *abp4*, *PIN1a* expression was elevated in mesocotyls grown in red light conditions. Knockout of both, *ABP1* and *ABP4* resulted in increased expression of *PIN1a* in blue light, but in normal transcript level in red light. Our data led to the conclusion that functional ABP1 contributes to the stimulation of *PIN1a* expression in red light, but to the inhibition of *PIN1a* expression in blue light conditions. Interestingly, ABP4 seems to function exactly in the opposite way.

Our data show that in light-dependent manner, ABP1 and ABP4 play a role in the expression of polar auxin transport gene *PIN1a*, and bring more information about the interaction between the light and auxin signaling in plant growth and development in maize.

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# ABSCISIC ACID MEDIATED REGULATION OF SUCROSE TRANSPORT AND HYDROLYSIS DURING GRAIN FILLING IN WHEAT

**Mukherjee S, Liu A, Deol KK, Ayele BT**

Grain filling and yield are regulated partly by the translocation of assimilates from source tissues, and sucrose transporter proteins play roles with this respect. Genes encoding sucrose transporter proteins (SUTs) have been identified from several species including cereal crops such as rice and wheat. Recently we identified and functionally characterized the three homologues of a new SUT gene, designated as *TaSUT2*. While *TaSUT1* is reported to be plasma membrane, our analysis showed that *TaSUT2* is localized to the tonoplast; *TaSUT1* is highly expressed during grain filling. Sucrose imported to the developing endosperm is mainly hydrolyzed by sucrose synthase (SuSy) to provide hexose sugars that are used as substrates for the synthesis of starch, the major storage reserve in wheat grains. Among the wheat *SuSy* genes, *SuSy2* appears to be the major contributor of SuSy activity. To gain insights into the ABA mediated transcriptional regulation of sucrose transport and hydrolysis, we examined the responsiveness of genes related to these processes in developing grains. Our study showed ABA induced repressions of *TaSUT1* and *TaSuSy2*, implying that ABA negatively regulates sucrose import into the endosperm and its hydrolysis.

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# COPPER-INDUCED CHANGES IN NET H<sup>+</sup> AND K<sup>+</sup> FLUXES OF *SILENE PARADOXA* ROOTS

**Palm E<sup>a</sup>, Colzi I<sup>a</sup>, Gonnelli C<sup>b</sup>, and  
Mancuso S<sup>a</sup>**

Despite the increasing number of studies on the physiology of metallophytes, the mechanisms of metal tolerance in higher plants are still poorly understood. Here we propose a comparative investigation on copper-induced net flux rates of hydrogen (H<sup>+</sup>) and potassium (K<sup>+</sup>) of intact roots after short-term exposure to 5 μM copper (Cu<sup>2+</sup>) in three contrasting populations of hydroponically-grown *Silene paradoxa*, measured with non-invasive and ion-selective vibrating microelectrodes. Such populations were from an uncontaminated soil (metal sensitive), a mine dump (Cu-tolerant) and a serpentine soil (Ni-tolerant). Preliminary data showed an increase in net H<sup>+</sup> influx of Cu-tolerant roots in response to the addition of Cu to the measuring solution, as compared to the metal-sensitive and serpentine populations in which no change in the direction or rate of H<sup>+</sup> was observed. Net K<sup>+</sup> flux changed from influx to efflux in the sensitive roots while the opposite response was observed for Cu-tolerant roots, and the rate of K<sup>+</sup> efflux increased in the serpentine roots. The change in the net fluxes of H<sup>+</sup> and K<sup>+</sup> suggests that the plasma membrane of *S. paradoxa* roots could play a role in tolerance to Cu that includes active transport of Cu out of the roots, a mechanism that has been observed in Cu-tolerant populations of *Silene vulgaris*. We propose flux measurements in response to long-term exposure to copper to determine whether the activity of the plasma membrane in *S. paradoxa* roots is a constitutive response or one that is up- or down-regulated over time.

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# COMPETITION AND COOPERATION IN *PISUM SATIVUM* L. IN RELATION TO GENETIC PROXIMITY AND NUTRIENTS AVAILABILITY

**Pezzola E, Masiero F, Pandolfi C & Mancuso S**

It has been found that the plants characterized by a viscous population evolved specific mechanisms to recognize the genetic proximity of their neighbors. This, in most of the cases, results in a cooperating behavior among relatives (i.e. a reduced competition) which allows them to increase their gains, in a so called "extended fitness" as theorized by Hamilton in 1964.

In presence of a competitor plants allocate more organic matter towards competitive traits, to reduce the resources of the competitor or to limit the effect of the competitor on resources uptake. In addition, thanks to phenotypic plasticity, plants cope with environmental issues by altering their growth model depending on limiting resources.

The objective of this study is to understand if kin selection is a constant behavior or it is influenced by environmental conditions (i.e. nutrient unbalance).

*Pisum sativum* L., was grown at two different nutritional levels and in association with kin or non-kin individuals to evaluate the interaction of these two factors on their growth models.

We found that relative growth rate (RGR) and fruit production are both affected by their neighbors. In particular, fruit production remains unvaried when plants are grown with kin, in both nutritional conditions, and are significantly reduced when grown with non-kin at low nutrition.

These preliminary results suggest that *Pisum sativum* may behave differently towards kin depending on the nutritional status, showing that plants are able not only to adjust their competitive strategies according to the identity of their neighbors but also to integrate these information with the environmental condition.

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# SIZE-DEPENDENT ECOTOXICITY OF BARIUM TITANATE PARTICLES: THE CASE OF *CHLORELLA VULGARIS* GREEN ALGAE

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## Abstract

Studies have been demonstrating that smaller particles can lead to unexpected and diverse ecotoxicological effects when compared to those caused by the bulk material. In this study, the chemical composition, size and shape, state of dispersion, and surface charge, area and physicochemistry of barium titanate (BT) in two different sizes were characterized. We also used the green algae *Chlorella vulgaris* grown both in artificial and natural culture medium (Bold's Basal, BB, or Seine River water, SRW, respectively) as a model organism to assess, for the first time, its aquatic toxicology. Responses such as growth inhibition, cell viability, superoxide dismutase activity, adenosine-5-triphosphate content and photosynthetic activity were evaluated. Tetragonal BT (~170 nm, 3.24 m<sup>2</sup> g<sup>-1</sup> surface area) and cubic BT (~60nm, 16.60 m<sup>2</sup> g<sup>-1</sup>) particles were negative, poorly dispersed, readily aggregated, and precipitated in both SWR and BB medium. As for the aquatic toxicology: (i) BT has a statistically significant effect on *C. vulgaris* growth even at the lower concentration tested (1ppm), what seems to be mediated by induced oxidative stress caused by the particles; (ii) the BT behavior was different when in synthetic or in natural culture media, the toxic effects in *C. vulgaris* being more pronounced when grown in SRW (in this case, a worse physiological state of the algae growing in SRW can occur and account for the lower resistance, probably linked to a paucity of nutrients or even to a synergistic effect with a contaminant from the river); and (iii) size does not seem to be an

issue influencing the toxicity in BT particles toxicity since micro- and nano-particles produced significant effects on algae growth – although the growth inhibition was more pronounced with the nanomaterial.

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# MOLECULAR AND CELLULAR MECHANISMS OF PEA SYMBIOTIC NODULE DEVELOPMENT

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We have studied different aspects of nodule development of pea wild-type and symbiotic mutants including: reorganization of the tubulin cytoskeleton; production of reactive oxygen species; and functioning of the antioxidant system; as well as senescence. The three-dimensional microtubular organization of each nodule histological zone in pea nodules was analyzed and linked to the developmental processes during nodule cell differentiation. This study has revealed the important role of endoplasmic microtubules in the growth of the infection thread, the formation of the infection droplet and bacterial release into the host cell cytoplasm as well as in the orientation of bacteroids. It was also observed that rhizobial infection triggers an alteration in the specific orientation of cortical microtubules, which is characteristic for adjacent cells that remain uninfected. It was shown that hydrogen peroxide is involved in maturation of the infection thread wall and release of bacteria into the host cell cytoplasm from infection droplets.

It was demonstrated that in symbiotic nodules the antioxidant system is actively functioning; moreover, glutathione is one of the most important components. For analysis of pea symbiotic nodule senescence, we examined transcriptional activity using a set of molecular markers based on the genes of cysteine proteases (*PsCyp1*, *PsCyp15a*) and thiol protease (*PsTPP1*); the gene of transcriptional factor bZIP (*PsATB2*); the gene of gibberellic 2-8-oxidase (*PsGAOx2*); the genes of 1-aminocyclopropan-1-carboxylate synthetase and oxidase (*PsACS2*, *PsACO1*);

and the gene of aldehyde oxidase 3 (*PsAO3*). The increased expression of these analyzed genes correlated with the increase of nodule age for nodules of wild-type and for mutants with the early senescence phenotype.

The use of laser micro-dissection revealed the increase of expression of analyzed genes in infected cells that demonstrated morphological features of senescence in comparison with cells in which such features were absent.

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# **INACTIVATION OF PLASMA MEMBRANE H<sup>+</sup>-ATPASE AS A PROBABLE MECHANISM OF VARIATION POTENTIAL INFLUENCE ON PHOTOSYNTHESIS**

**Sherstneva O., Surova L, Morozova E, Gaspriovich V and Sukhov V**

Damaging stimuli induce variation potential (VP) that causes functional changes in higher plants, including photosynthetic response. It is known that VP can induce short-term inactivation of photosynthesis; however, mechanism of VP influence on photosynthetic processes is still discussed. The aim of our study was to analyze H<sup>+</sup>-ATPase participation in VP influence on photosynthetic processes in pea plants. It has been shown that burn-induced VP reduced CO<sub>2</sub> assimilation rate, decreased photosystem I and II quantum yields and raised non-photochemical quenching of fluorescence in pea. Ratiometric measurement of pH changes using pH-sensitive fluorescent dyes showed that VP generation was accompanied with pH increase in apoplast and pH decrease in cytoplasm that implies probable inactivation of plasma membrane H<sup>+</sup>-ATPase. Pea leaf was incubated in sodium orthovanadate and fusicoccin to analyze the influence on photosynthetic response of H<sup>+</sup>-ATPase inhibition and activation, respectively. Pretreatment by orthovanadate decreased VP amplitude and reduced substantially the photosynthetic changes. Treatment by fusicoccin did not affect VP parameters, but increase the amplitude of non-photochemical quenching rise. Thus inactivation of plasma membrane H<sup>+</sup>-ATPase may be considered as a mechanism of VP-induced photosynthetic response in pea plants. Investigation of sodium orthovanadate influence on photosynthetic parameters in isolated protoplasts showed that orthovanadate increased non-photochemical quenching

and decreased photosystem I and II quantum yields. Thus H<sup>+</sup>-ATPase inhibition is important mechanism of VP influence on photosynthesis. This influence is probable to be connected with pH changes in apoplast and cytoplasm. Apoplastic pH increase possibly changes carbonic anhydrase and (or) aquaporins activity and CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> ratio. Influence of pH decrease in cytoplasm on photosynthesis can be caused by chloroplast stroma and lumen acidification that can inactivate Calvin cycle enzymes, change ferredoxin-NADPH oxidoreductase localization and activity and enhance non-photochemical quenching of fluorescence. This work was supported by the Russian Scientific Fund (Project No. 14-26-00098).

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# **ER-PM-LOCALIZED SYNAPTOTAGMIN 1 AND TGN/ EE-LOCALIZED SYNAPTIC VESICLE PROTEIN 2-LIKE IN ENDOCYTOTIC VESICLE RECYCLING PATHWAYS OF ARABIDOPSIS ROOT APEX CELLS**

**Siao W, Voigt B and Baluška F**

Arabidopsis synaptotagmin 1 (SYT1) has recently been shown to be an ER-PM contact site component and is well-known to function in abiotic and biotic stress responses. However, the function of SYT1 on the ER-PM contact sites remains unclear. Our data showed that SYT1 was localized on the ER and attached to the PM at the SYT1-specific ER-PM contact sites. The SYT1-specific contact sites are distinguishable from, and surrounded around, the VAP27-localized contact sites. We also demonstrated that root cells of the *syt1* and *syt1/3* mutants have smaller BFA-induced compartments in the transition zone, indicating that endosomal recycling pathways are modulated by SYT1 and SYT3. Arabidopsis Synaptic Vesicle Protein 2-Like (SVL) has been shown to be a nicotinic acid and trigonelline transporter when expressed in *L. lactis* cells. Our data demonstrated that SVL is localized to trans-Golgi network (TGN) and early endosomes (EE) in Arabidopsis root apex cells. This report focuses on roles of Arabidopsis SYT1 and SVL in the endocytic vesicle recycling pathway. We are discussing the physiological aspects of these proteins concerning the signal transmission in control and stressed roots.

We report that *SVL* mutants showed different inhibition behavior to the high concentration of nicotinic acid treatments comparing with the wilt type. Further investigation on possible functions of SYT1 in the formation of ER-PM contact sites, and SVL in endocytosis and endocytic recycling will provide better understanding on neuronal-like signal transmission mediated by plant endosomes and endocytic vesicle recycling in root apices.

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# **INFLUENCE OF VARIATION POTENTIAL ON DAMAGE OF PHOTOSYNTHETIC MACHINERY TO HEATING IN PEA**

**Sukhov V, Surova L, Sherstneva O,  
Mudrilov M and Vodeneev V**

Variation potential (VP) is induced by local wounding and causes numerous functional responses in plants. Increase of plant resistance to stressors is possible result of the responses, but this hypothesis requires experimental investigation. We investigated influence of burning-induced VP on damage of photosystem I (PSI) and photosystem II (PSII) under heating in pea. System for photosynthetic analysis (GFS-3000 and Dual-PAM-100, Heinz Walz GmbH, Germany) was used for the investigation. It was shown that VP induced decreased PSI damage and increased PSII damage under heating to 53°C in 15 and 45 min after variation potential propagation. Increased PSII damage was connected with low transpiration rate in experimental plants that intensified heating of leaf under high external temperature. Decreased PSII damage was connected with inactivation of photosynthesis which had been induced by VP before heating. There were several potential mechanisms of VP-induced photosynthetic response influence on resistance of PSI to heating. (i) VP-induced growth of non-photochemical fluorescence quenching which was greatly increased after variation potential propagation. It is known that the quenching protects PSI and PSII under stressors. (ii) VP-induced growth of cyclic electron flow through PSI. Increase of cyclic electron flow is known to be important mechanism of photosynthetic machinery protection.

We showed that VP intensified the cyclic electron flow in about 10 min after electrical signal propagation, and this response was connected with inactivation dark stage of photosynthesis. (iii) VP-induced growth of ATP content in leaf because it is known that ATP can participate in increase of photosynthetic machinery resistance to stressors. Our results showed that VP induced two transient increases in ATP content (10 and 40-50 min after electrical signal propagation). Thus VP can increase PSI resistance as well as stimulate PSII damage under heating.

This work was supported by the Russian Scientific Fund (Project No. 14-26-00098).

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# THE INVOLVEMENT OF A WALL ASSOCIATED KINASE IN THE RESPONSE TO GRAVISTIMULATION IN POPLAR STEMS

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Plants are able to sense organ tilt and reorient their growth according to the gravity vector. In angiosperm woody species, the motor for the stem straightening in parts undergoing cambial growth is differential maturation between the two sides of the stem. The dynamics of the gravitropic response involved the sensing of the local inclination angle (gravisensing) as well as the sensing of the local curvature which progressively takes place in the straightening process [Bastien et al (2013)]. If one wants to specifically address the question of the gravisensing / graviresponse in plants undergoing secondary growth, the gravistimulation should not induce organ deformation. This is why we chose to tilt staked poplar trees in our experimental design.

From this, one challenging research question is the identification of molecular actors specifically involved in gravisensing / graviresponse. Since it has been proposed that plant cells could sense gravity through the cytoskeleton-plasma membrane-cell wall continuum, wall associated kinases (WAK) with an extracellular part that can bind to pectins localized in the cell wall, appear as molecular candidates.

Using *in silico* approaches, we showed that the WAK family in poplar is the largest characterized to date with 175 sequences. Gene expression was analyzed in various organs and tissues. Most WAKs were weakly expressed. Only 28 WAKs were expressed in the stem. One WAK is of particular interest showing differential expression after tilting of the stem. The protein was localized in young xylem and bark cells devoid of amyloplasts. These data suggest that a WAK could be involved in the sensing/response to gravistimulation in a manner independent of the displacement of amyloplasts which is behind the starch-statolith hypothesis [Sack, 1997].

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Sack (1997) Planta 203, S63-S68

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# STRIGOLACTONES: SIGNAL REGULATORS BETWEEN PHOSPHORUS AND ETHYLENE INTERACTION

**Torres-de los Santos R, Albores-Flores V, Ovando I, Salvador-Figueroa M and Adriano-Anaya M. L**

Strigolactones (SLs) are recently reported like signal molecules that regulate many physiological plant pathways, like root and shoot branching, nutritional acquisition and control the biotic root interactions. SLs production mechanisms are not completely unknown, nonetheless there are some reports that involve ethylene and phosphorus signaling pathways. In this study, we described for the first time in mutant tomato plants the *Carotenoid Cleavage Dioxygenases (CCD) 7* and *8* gene expression (SLs biosynthesis genes) and putative SLs transporter gene (*LsPDR1*). We used genotypes with altered ethylene biosynthesis (*epinastic*, *epi*, ethylene overproducer) and perception (*rin*, fail to produce high levels of ethylene and to repining in response to exogenous ethylene) pathways treated with a low (100 ppm) and high (300 ppm) phosphorus concentrations. Real-time qPCR was based on Ct values, normalized with the household gene *LsEF-1a* (X14449). *CCD7* and *CCD8* transcript accumulations in wild type plants were negatively regulated in both levels of phosphorus applied. In mutant plants, level expressions were directly related to the genotype for all genes. In *rin* plants as compared to wild-type plants, *CCD7* and *8* expressions were inhibited showing no relationships Pi-SLs. In the *epi* mutant, their expressions were induced. An ethylene response constitutive block Pi negative action on the production of SLs, and therefore demonstrates that at least part of antagonism Pi/ethylene is mediated by the SLs. The expression of *LsPDR1* was genotype and phosphorus concentration dependent.

Transporter expression was enhanced in *Epi* under conditions of Pi. This suggests that the *Epi* mutation interacts with phosphorus to enhance expression of the gene *LsPDR1*, and probably, transporting strigolactones.

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# ANTAGONISTIC PIGMENTATION IN LETTUCE LEAVES UNDER IRRADIATION BY HB-LEDS WITH BROAD RANGE OF BLUE AND RED SPECTRA

**Ueda K, Kawano T**

Under sunlight, leaves of lettuce (*Lactuca sativa* L., cv. Lollo Rossa) often develop a characteristic reddish blue color representing the biosynthesis of anthocyanin. However, under artificial light sources such as halogen lamp and sodium lamp, lettuce leaves show only partial or none development in leaf color, possibly due to the lack of specific light components stimulating the plants.

Here, we attempted to determine the light components required for induction of anthocyanin biosynthesis in young lettuce leaves (3 weeks after seeding) under controlled illumination for 3 days, using high-brightness (HB)-light emitting diodes (LEDs) with various spectral features. The wavelength of light examined here include 365, 375, 420, 430, 450, 470, 505, 525, 625, 645, 660 and 735 nm. As negative control, a group of plants was incubated in the darkness for 3 days.

Prior to and after the lighting treatments, leaf color composition was determined using a hand-held Hunter's colorimeter (CR-13, Konica Minolta, Tokyo, Japan) and expressed with CIELAB system in which  $L^*$ ,  $a^*$ , and  $b^*$  values represent the lightness, the green-red color scale (positive, red; negative, green), and the blue-yellow color scale (positive, yellow; negative, blue), respectively.

We have specifically focused on the changes in  $a^*$  and  $b^*$  values and the data points were plotted on the 2D-coordination plane with  $a^*$  and  $b^*$  axes. In the preliminary experiments, correlation between CIELAB values (especially  $a^*$  value) and anthocyanin content was obtained.

Action spectra obtained here suggested that the leaf coloring (reddening and de-greening) can be stimulated by a broad range of blue light (420-470 nm) peaking at 450 nm. On the other hand, red light (625, 645, or 660 nm) applied solely drastically induced the re-greening and de-reddening of the leaves. In addition, the red range of light effectively opposed the action of blue lights in pigmentation. Interestingly, green light (525 nm) behaved both positively and negatively depending on the intensity of light, suggesting that the ranges of light perception by blue-responsive and red-responsive mechanisms overlaps at the green region.

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# PURPLE MOOR GRASS INDUCES A RAPID DECREASE OF PHOTOSYNTHESIS IN YOUNG OAK AFTER FOREST CLEAR-CUTTING

**Vernay A<sup>1,2</sup>, Malagoli<sup>1,2</sup> P, Guinard<sup>1,2</sup> L,  
Améglio<sup>2,1</sup> T and P Balandier<sup>3</sup>**

The purple moor grass (*Molinia caerulea* (L.) Moench) is a well-known resource competitor to the detriment of tree regeneration in many boreal or temperate forests of the Northern hemisphere. This study aimed at investigating to what extent soil nitrogen capture in interaction with light availability drives the early establishment of competition between oak (*Quercus petraea* (Matt.) Liebl.) and *Molinia* seedlings. Two-year-old oak was grown in 20 l pots, alone or in combination with *Molinia*, for two levels of light availability (11 and 55% of incident photosynthetically active radiations) in a greenhouse. Leaf photosynthesis measurements and soil <sup>15</sup>N-labelling were used to monitor changes in carbon assimilation and soil nitrogen uptake between and within species under well-watered conditions. Presence of *Molinia* had no significant effect on short-term oak seedling growth regardless of the light availability. However, increase in incident light resulted in the rise of both nitrogen uptake and photosynthesis capacity in *Molinia*. Meanwhile, N taken up by oak grown with *Molinia* under unshaded environment hardly changed but photosynthesis capacity strongly decreased when compared to shady conditions. In conclusion, these results pointed out a short-term response of photosynthesis and N capture in mixture of *Molinia* – oak seedlings in unshaded conditions (*i.e.* after forest canopy opening), which might partly result from a larger nitrogen capture by *Molinia*.

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# ESTABLISHMENT OF CALCIUM-INTOLERANT SPHAGNUM MOSSES IN ALKALINE PEATLANDS

Vicherová E, Hájek T

Although *Sphagnum* mosses are generally calcifuges they recently expand to endangered habitats of alkaline [calcium bicarbonate-rich] fens and convert them to acidic, *Sphagnum*-dominated poor fens with low species diversity.

We assessed the expansion potential of three *Sphagnum* species using field transplantation experiments with the respect to water chemistry and availability: individual *Sphagnum* shoots were positioned along the natural hummock-hollow gradient of water-table depth. The shoot survival was evaluated regularly for 3.5 years. The physiological mechanisms behind calcium toxicity were surveyed by a series of laboratory cultivation experiments.

All transplanted species survived at all localities, even in calcareous fens where no sphagna had occurred before. However, shoot mortality was generally high; only few individuals survived in very alkaline fens (up to 100 mg Ca<sup>2+</sup>/L and pH 8) but the survival exceeded 50% in moderately alkaline fens.

Water chemistry (flooding, pH, [Ca<sup>2+</sup>]) controlled *Sphagnum* survival during the first experimental months. However, the long-term survival and expansion was, in addition to water chemistry, determined by local climate. Consequently, shoots of all species expanded to small *Sphagnum* patches in the most humid localities.

Our cultivation experiments revealed insufficient control over the balance of intracellular Ca<sup>2+</sup> uptake/efflux in the calcifuges. We refused hypothesized mechanism of calcium toxicity involving Ca<sup>2+</sup>-oversaturation of cell-wall cation-exchange sites.

To conclude, calcifuge mosses did not evolve sufficient control over protoplasmic Ca<sup>2+</sup> balance. Their long-term survival and expansion in alkaline fens is thus limited to less calcareous microhabitats (hummocks) and facilitated in humid areas with lower desiccation risk.

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# **INTEGRATING PROTEOMICS AND ENZYMATIC PROFILING TO DECIPHER SEED METABOLISM AFFECTED BY TEMPERATURE INDUCED SEED GERMINATION**

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D<sup>3</sup>, Gibon Y<sup>3</sup>, Bailly C<sup>1</sup>, Corbineau F<sup>1</sup>,  
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Temperature is an important environmental factor affecting seed dormancy and germination. The mechanism by which temperature induces germination is however still unclear. Proteomic study has been performed in dormant sunflower seeds during imbibition at permissive and non-permissive temperature for germination, 20°C and 10°C, respectively. Proteomic study was completed by polysome and proteasome assessment and enzymatic profiling on several altered proteins mainly involved in metabolism and energy. Results showed that 20°C induced the activation of both protein synthesis and degradation processes, the latter being probably more active to tip the balance towards a decrease in protein abundance. More importantly, enzymatic profiles do not correlate to protein abundance at 20°C suggesting that post-translation modifications (PTMs) such as oxidation were likely to be associated to germination potential. Enzymatic activity data allowed clarifying seed metabolism affected by temperature over imbibition time. Enzymatic profiling seems to be necessary to gain insights into molecular networks controlling seed dormancy and germination.

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# ANAESTHESIA AND PLANTS

**Yokawa K<sup>1,2</sup>, Kagenishi T2, Mancuso S<sup>3</sup>,  
and Baluška F<sup>2</sup>**

Anaesthesia is essential for many medical treatments, especially surgery. In fact, humans have been using natural chemical compounds causing hallucination for religious or other purposes in recorded history. Anaesthesia for medical purposes was introduced only in the 19th century. Many kinds of different chemicals are known to induce the anaesthesia effects (diethyl ether, chloroform, halothane, isoflurane, xenon). However, the exact mode of the anaesthetic action is still unknown. The one of deep mysteries is how these different compounds with different chemical structures, even inert elements such as xenon, can act as anaesthetic agents inducing loss of consciousness. Besides recent receptor-hypotheses, other hypotheses were proposed such as the Meyer-Overton theory, based on a relationship between lipid-solubility (namely cellular lipid bilayer) of these compounds and the magnitude of anesthesia loss. Here, we are studying the effects of both general and local anaesthetics (diethyl ether, xenon, lidocaine) on roots and plants. Obtained results indicate that gaseous anesthetics such as diethyl ether and xenon inhibit chlorophyll biosynthesis and seed dormancy breaking in both cress and maize. Moreover, ROS generation and cytoplasmic Ca<sup>2+</sup> elevations are induced in Arabidopsis and maize root cells after diethyl ether treatment. Our findings suggest that these anesthetics alter cellular membrane properties. Remarkably, the application of lidocaine, well-known local anaesthetic, to only root part inhibits Mimosa leaf-closing movements after their mechanical stimulation.

These results provide important clues to our very limited understanding of the anaesthetic actions on both the animal/human nervous systems as well as on the living systems.

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# **COMPARISON OF SALT STRESS EFFECTS ON GROWTH CHARACTERISTICS AND ACTIVITY OF ANTIOXIDANT ENZYME OF CHICKPEA (*CICER ARIETINUM* L.) AND REDROOT PIGWEED (*AMARANTHUS RETROFLEXUS* L.)**

**Zamani GR, Doraki GR and Sayyari MH**

Salinity is one of the main problems in arid and semi-arid region that affect the growth of plants. This stress in plants causes disorder of ions absorption and the osmotic stress. Subsequently leads to oxidative stress by increasing the active oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals. The most immediate solution for the plant to overcome the effects of oxidative stress, activation is antioxidant enzymes. In order to study the effect of salinity stress on physiological and morphological characteristics of chickpea and redroot pigweed and activity of antioxidant enzymes, an experiment was conducted in the research greenhouse, University of Birjand in 2013 as a Randomized Complete Block design (RCB) with four replications. Salinity levels were 1, 3, 5, 7 and 9 dS/m and chickpea cultivar was AZAD. Results showed that salinity has significant effect on plant height, leaf area, total dry weight, relative water content (RWC), relative permeability of the membrane and antioxidant activity enzymes. The results of this experiment showed that salinity reduces the height of the chickpea and redroot pigweed 22.44 and 10.22 percent, respectively. Also, both of leaf area and plant dry weight decreased by increasing salinity levels.

In this experiment with increasing of salinity from 1 to 9 dS/m, leaf area of chickpea and redroot pigweed reduced 58.36 and 13.43 percent, respectively. Also dry matter production of chickpea and redroot pigweed in highest salinity level 39.4 and 31.3 percent was reduced than the lowest levels, respectively. Relative water content (RWC) in the highest salinity level than the lowest salinity stress in chickpea and redroot pigweed respectively 13.52 and 6.57 percent was reduced.

Also, relative membrane permeability in chickpea and redroot pigweed in highest salinity stress than the lowest, 31.2 (At 7dS/m) and 62.69 percent was increased, respectively. The plant antioxidant enzymes activity in this experiment were affected by salinity, as in chickpea, activity of catalase, superoxide dismutase and ascorbate peroxidase in the highest activity level than the lowest 63.79, 57.27 and 75.95 percent were increased, whereas, in redroot pigweed were increased 80.80, 63.18 and 84.14 percent respectively. In this experiment, in 9 dS/m treatment, the activity of catalase and superoxide dismutase of pea decreased.

Keywords: Antioxidant enzymes, Leaf area, Relative water content, relative membrane permeability

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# ROLE OF ZINC IN ALLEVIATING CADMIUM TOXICITY IN *CHARA AUSTRALIS*

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Characean algae have been proposed for use in phytoremediation of heavy metals because of their high biomass and cation uptake capacity. We previously showed that *C. australis* can take up cadmium up to 72 mg (kg DW)<sup>-1</sup>. Since effective phytoremediators must be resistant to metal toxicity, we explored Cd toxicity in *C. australis*. We showed that 2 ppm (18 μM) Cd in solution is toxic, but adding 5 ppm (76 μM) zinc decreased mortality by 8 to 41%. Both treatments resulted in equivalent Cd uptake, so decreased Cd concentration was not the cause of reduced mortality. Heavy metals cause an increase in damaging reactive oxygen species (ROS) in other systems. We therefore hypothesized that Cd induces an ROS increase, which is limited in the presence of Zn due to increased glutathione. Glutathione plays an important role in controlling the redox potential of the cytoplasm by shifting between its reduced and oxidized forms (GSH and GSSG) and therefore altering ROS levels. Between 0.5 and 24 h, ROS in metal-stressed cells was always less than controls, with values ranging between 33% and 6.5% of controls. Neither GSH nor GSSG was different than control in the first 4 hours of exposure. However, at 24 h, GSH increased in Cd-stressed plants (12 X control). With Cd + Zn, the increase in GSH (32 X control at 24 h) was greater than in the Cd-stressed system. GSSG did not increase in plants treated with Cd only, but increased by 350% over controls with Cd + Zn.

This suggests that Zn effects on Cd toxicity are mediated by GSH, but not necessarily due to its effect on ROS.

We explore alternative theories for the role Cd and GSH in reducing Cd toxicity.

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# THE SOCIETY OF PLANT SIGNALING AND BEHAVIOR

serves the community of scientists interested in sensory plant biology, signaling, and communicative ecology in plants.

It fosters transdisciplinary interactions among plant molecular biologists, cell biologists, plant physiologists and plant ecologists.

The goal of this field is to illuminate the structure of the information network that exists within plants. Plants are dynamic and highly sensitive organisms that actively and competitively forage for limited resources both above and below ground. Plants accurately compute inputs from the environment, use sophisticated cost-benefit analysis, and take action to mitigate diverse environmental insults. Plants are also capable of refined recognition of self and non-self, and are territorial in behavior. This view sees plants as information processing organisms with complex, long-distance communication systems within the plant body and extending into the surrounding ecosystem. Our Society was originally founded in 2005 as the Society for Plant Neurobiology to reflect these views of plant function. In May 2009 the Society voted to expand its view and change its name accordingly.

One goal of establishing a community for Plant Signaling and Behavior is to provide a venue for all interested biologists to explore complex plant behavior utilizing all levels of experimental approach.

Among our symposia participants have been molecular geneticists, biochemists, electrophysiologists, physiological ecologists, community ecologists, mathematical modelers, plant designers, and even philosophers. Plant Signaling and Behavior will use the lens of integrated signaling, communication, and behavior to integrate data obtained at the genetic, molecular, biochemical and cellular levels with physiology, development and behavior of individual organisms, plant ecosystems and evolution.

**For more information on the society please consult our web site:**

**<http://www.plantbehavior.org>**



## THE FRENCH SOCIETY OF PLANT BIOLOGISTS (SFBV)

formerly the French society of plant physiologists was created in 1955. The SFBV count approximately 400 members belonging to several universities and national institutes of research (INRA and CNRS). The goals of the SFBV are: To promote the engagement of students in plant biology.

To facilitate the relations between scientists working in several institutions and areas of plant biology in France and abroad.

To be a representative organ of French plant biologists in France and abroad.

To promote and explain if necessary to the society, via press media and / or vulgarization conferences the benefits of the research in plant biology and biotechnology.

The SFBV organizes an international congress for plant biologists each two years. The SFBV organizes as well meetings for young scientists and thematic workshops.

**For more information on SFBV please consult our web site:**  
<http://sfbv.snv.jussieu.fr>



# UNIVERSITÉ PARIS DIDEROT

In 1970, after the events of May 1968 and in the context of the application of the Faure law, the University of Paris was divided into 13 independent universities. Paris 7 was unique in that the university could already offer a fairly wide range of courses in several subject areas: human sciences, science and health, etc. In 1994 Paris 7 chose for Denis Diderot, the great philosopher of the enlightenment as its emblematic figure to reinforce and promote its multidisciplinary projects. Paris 7 university then adopted the name "Paris7-Denis Diderot" university. In 2007, Paris Diderot moved from its initial site, Jussieu campus in the Latin district of Paris 5, to the current site at Paris Left Bank in the south east of the French capital city. The move near the Bibliothèque Nationale de France (BNF – National French Library) provided Université Paris Diderot with new facilities and boosted its ambition to be a leading multidisciplinary university.

For more information on Université Paris Diderot please consult our web site: <http://www.univ-paris-diderot.fr/english>

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## NAKED PLANTS

Naked Plants is the result of an art project that lasted two years, growing plants and photographing them completely denuded, roots exposed but still alive, suspended for a few moments in real locations (not in a studio) and then replanted in their natural habitat (no plant has been lost in this project).

This research shows through images that plants have their own lives and their own personality and intelligence. Shooting in public places and familiar settings, they overlap the image of man and replace him temporarily in an unreal vision that shows that we depend on their lives and derive from their development. We share with them 26% of the genetic code. Through this photographic project, the author Gianluca Balocco compares the time of humanity with that of plants.

Naked Plants is also a book published by Aboca Editore Italie (160 pages color) with texts from: Elio Grazioli. Riccardo Panattoni. Carla Balocco. Michael Pollan. Stefano Mancuso. Clark Lawrence. Swami Joythimaiananda Pietro Leemann. Gianluca Balocco.

*Web: [www.gianlucabalocco.com](http://www.gianlucabalocco.com)*

### **GIANLUCA BALOCCO**

Artist, Photographer, Writer, Architect

### **THE RESEARCH**

Even though western culture is pervaded by millions of images, I believe photography (still very young) hasn't yet exhausted its expressive and cultural potential. Photography, like thought, has a neutral dimension and as such manifests itself only when it meets reality. This medium, like our eyes, is nourished by images and aligns itself perfectly with the mental and neuronc functioning of the human mind. Photography is first and foremost an autonomous language in which images, like words, become the third abstract dimension of a real spatial and temporal field.

Photography is not a translatable language (it can only be described or interpreted) and it can only be partially considered a "universal language". I believe that photography finds its own artistic dimension in the moment in which it fulfils its own abstract codes. In the field of thought defined by image/space/time, our mind is regularly nourished by images and produces its own at such a rate that a very fine line divides experience from imagination and emotion. I would like to add a final consideration about photography: it is an irreplaceable language in the evolution of humankind, a means of understanding both ourselves and the world. In these last few years of research, I've become interested in psychology and sociology. I've always been attracted to everything dealing with the mind, thought, the subconscious, philosophy. I am convinced that it is in these areas of study that art can practice its collective mission.

# BIOGRAPHICAL NOTES

## GIANLUCA BALOCCO

In the early 1980's I studied photographic techniques and based my artistic research around this medium. In those years I considered photography as a simple means to attain a painterly result. The "photo traces", for example, are photographic images transformed into pictorial and material compositions that derive from a still frame of cinematographic film. Photography became, in this research, a quick and cold way to replicate the eye and the mind in the moment in which they capture a spatial and temporal moment. In these works the photographic image is not printed with a traditional process of developing on paper, but is created with a cast in plaster from a plastic and metal mould. In these works, photography becomes a simple material trace made of plaster, wool, and paper. In the 1990's my personal research in photography and video opened a new and important chapter exploring the mind of individuals: photographing the mind as a container of the collective memory. Our thoughts derive from images and translate into emotive and mnemonic states common to all. Fixed images of groups of people create a link between the external photographic vision and the internal mental image of each person who makes up a group. Travelling without a Passport is inspired by Bionian theories about the relationship between memory and emotions. In 1993 I presented the results of my work in the XLV Biennale di Venezia as a photography and video installation.

The work evolved from a three-month study of a group of 20 long-term female patients in a psychiatric hospital. A few years later I created a similar work with a group of poets who I met through the Modern Art Museum of Marseille. Some of my most important exhibits of the 1990's were in the following museums and galleries:

The Contemporary Art Museum of Umbertide, "Mystery and Myth" in the Fukuyama Museum of Art, Chiba Prefectural Museum of Art, and The Museum of Art - Kockhi, Ilda City Museum, "Five Rooms between Art and Depression (A. Bonito Oliva) at Museo Correr Venezia. In 1995, in collaboration with a group of psychoanalysts, I began researching photographic images as mental objects of a "collective thought". In 1996, I studied the theories of Wilfred Bion e di Gerald

Edelmann, and participated in several national conferences of psychoanalysts. In 1997 I was invited to create an installation at the Lingotto di Torino for the 100th anniversary of the birth of Bion. In the 1990s I directed the following short films: The Tube (1994), Poets don't cast shadows (1995), Brain Beat (1995), Rapid Eye Movements (1996), Twenty Five Thousand Days (1996), and October (1997). 2015. MAT-tam Isolo 17 Gallery. 2015 Fotografia Europea Reggio Emilia Biblioteca delle Arti.

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