Plant Signaling & Behavior

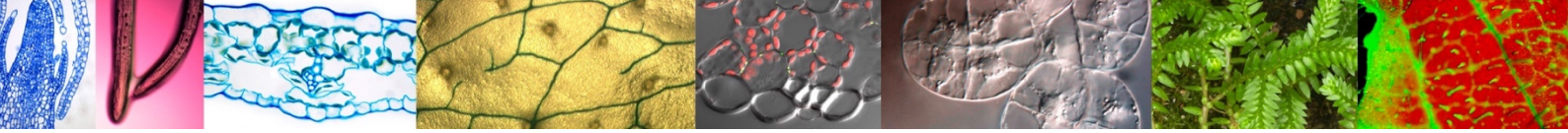
4th International Symposium

Proceedings



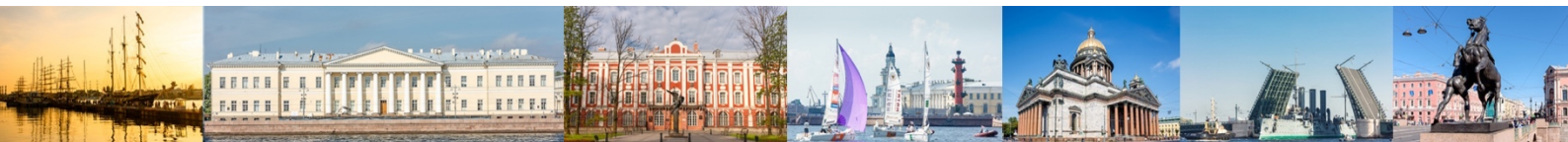
St. Petersburg, RUSSIA

19–24 June 2016



Proceedings of Fourth International Symposium on Plant Signaling and Behavior

St. Petersburg
SINEL Co.Ltd.
2016



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4th International Symposium on Plant Signaling and Behavior will be held from June 19 to June 24, 2016, at Komarov Botanical Institute of the Russian Academy of Sciences under the auspices of the Society of Plant Signaling and Behavior and the Russian Science Foundation. The scientific program includes plenary lectures by international speakers from Society of Plant Signaling and Behavior and Komarov Botanical Institute of the Russian Academy of Sciences. For early career and young scientists the School "Plant Stress Signaling" will be organized during the Symposium.

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Plant Signaling & Behavior

Dear Colleagues,

we are pleased to invite you to participate in the 4th International Symposium on Plant Signaling and Behavior.

The symposium will be held from June 19 to June 23, 2016, at Komarov Botanical Institute of the Russian Academy of Sciences under the auspices of the Society of Plant Signaling and Behavior and the Russian Science Foundation.

The plenary lectures will be given by leading scientists in diverse fields of plant signaling, ion transport, electro-physiology, photosynthesis, autophagy and programmed cell death. The symposium will provide an excellent opportunity for the synthesis of different views in the mutual attempt to develop concepts for the whole plant behavior and signal processing.

Second half of June is the best time to visit the city of St. Petersburg, to enjoy its world-famous museums and to contemplate the great architecture of the city in the mystic twilight of the White Nights.

The social program will include excursions to spectacular palaces and gardens of Saint Petersburg.

We are looking forward to seeing you!

On behalf of the organizing committees,

Vadim Demidchik and Olga Voitsekhovskaja



Komarov Botanical Institute
Founded in 1714

On behalf of the Society for Plant Signaling and Behavior, we welcome you to the Fourth International Symposium of the Society and to Saint Petersburg, Russia.

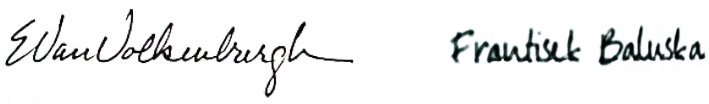
We are especially pleased to gather here during the summer solstice, where days are long and our discussions can be full and lingering. Our organizers have prepared several days of scientific sessions interspersed with social times and opportunities to see the sights of this fabulous city. New to this meeting is the inclusion of a School for Early Career and Young Scientists on "Plant Stress Signaling." Members of this school will attend talks and posters, and spend a day following the meeting discussing findings in greater depth, guided by speakers from the sessions. Special thanks to the Russian Academy of Sciences and the Russian Science Foundation for providing this opportunity.

Previous symposia of this Society have been held in Paris, New Delhi, and Vancouver, and under the name of the Society for Plant Neurobiology, in Italy, China, Slovakia, and Japan. We are excited to bring our research and scientific discussions to Russia, and to welcome travelers from other countries and continents to this spectacular venue.

Our meetings are dynamic, presentations are wide-ranging, and conversations uncover new ideas, collaborations, and questions to pursue. The focus is scientific discovery of the basis for plant behavior: how do plants sense their environment, integrate sensory information and encode responses to optimize their existence? Research will be presented addressing: plant behavior and ecology, electrophysiology, cell death and autophagy, complex adaptive and neuron-like reactions, interaction with bacteria and fungi, response to radiation and gravity, and signaling at all levels by hormones, neurotransmitters, under normal and stress conditions. The results inform fields not only in plant biology and agriculture, but also more generally neurobiology and behavior, ecology and evolution, philosophy, communication, and social sciences especially in those fields related to food, economic and environmental issues.

We hope that you enjoy the meeting, the formal presentations, informal exchanges, social times and spectacular field trips. Please let us know if we can help you make the most of your time here,

Elizabeth Van Volkenburgh and František Baluška



Plant Signaling & Behavior

Russian Science Foundation

Russian Science Foundation was established on the initiative of the President of the Russian Federation to support basic research and development of leading research teams in different fields of science. Legal status, powers, functions, proprietary rights and governance of the Foundation are determined by the Federal Law "On the Russian Science Foundation and Amendments to Certain Legislative Acts of the Russian Federation."

To achieve its goals, the Foundation selects science and technology programs and projects that fall under certain propriety categories, and does so on a competitive basis. Among these priorities are basic research initiatives by research groups or individual scientists, or members of the higher education teaching staff; development of scientific organizations and institutions of higher education, creation of world-class departments and laboratories in scientific organizations and educational institutions, development of experimental facilities for scientific research.

The governance structure of the Foundation is set up by the Federal Law: Supervisory Board, Management board and Director General. The Federal Law sets out a procedure for formation of these bodies as well as defines their authority. In order to provide the Foundation with the necessary expertise, the Federal law provides for creation of Review Panels acting as advisory bodies of the Foundation. Control over financial and economic activities of the Foundation is exercised by the Audit Committee of the Foundation. In accordance with the Federal Law, Russian Science Foundation submits the annual report for consideration of the President of the Russian Federation and the Government of the Russian Federation.



Dear colleagues,

It is a great pleasure to welcome you to the 4th International Symposium on Plant Signaling and Behavior which for the first time is hosted by the Komarov Botanical Institute of the Russian Academy of Sciences in Saint Petersburg.

The Komarov Botanical Institute is one of the oldest research institutions in Russia. It was established by the order of the Peter the Great as Physic Garden in 1714 and gradually developed into famous research centre of worldwide importance. We are proud that research on almost all fields of modern plant sceince is carried out in our institute.

In the 300 year history of the Botanical Institute, many plant physiologists have been working here exploring the mystery of plant life. The first position of "plant physiologist" was opened in the Imperial Botanical Garden (predecessor of the Komarov Institute) in 1843 and was taken by Karl Eugen von Mercklin, who studied plant anatomy, paleontology and developmental biology. He was followed by many distinguished scientists. The role of chlorophylls in shade and sun plants was studied by Vladimir Lyubimenko in the beginning of the 20th century. The violaxanthin (xanthophyll) cycle was discovered by David Sapozhnikov. Research in plant ecological physiology was provided by Oleg Zalensky, Olga Semikhatova and Yuri Gamalei who examined the structure and function of minor veins in over 800 species of dicotyledonous plants and developed a concept of endoplasmic networks uniting plant cells via plasmodesmata into whole plants like nervous system in animals.

The upcoming PSB Symposium is an important event for plant physiologists worldwide. This symposium is unique in bringing together eminent scientists from very different fields of plant science, in the mutual attempt to develop concepts for the regulation of whole plant behavior by the processing of diverse signals.

The second half of June is the best time to visit the city of Saint Petersburg, a cultural, architectural and esthetic jewel of Russia. You will have a unique possibility to explore it during what we call the "White Nights", when St. Petersburg is indeed magnificent.

I hope you will enjoy the very productive Symposium and your stay in Saint Petersburg!

Director of the Komarov Botanical Institute

V.T. Yarmishko


Начало экскурсий
MÖVENPICK





Symposium Venue and City Center Map

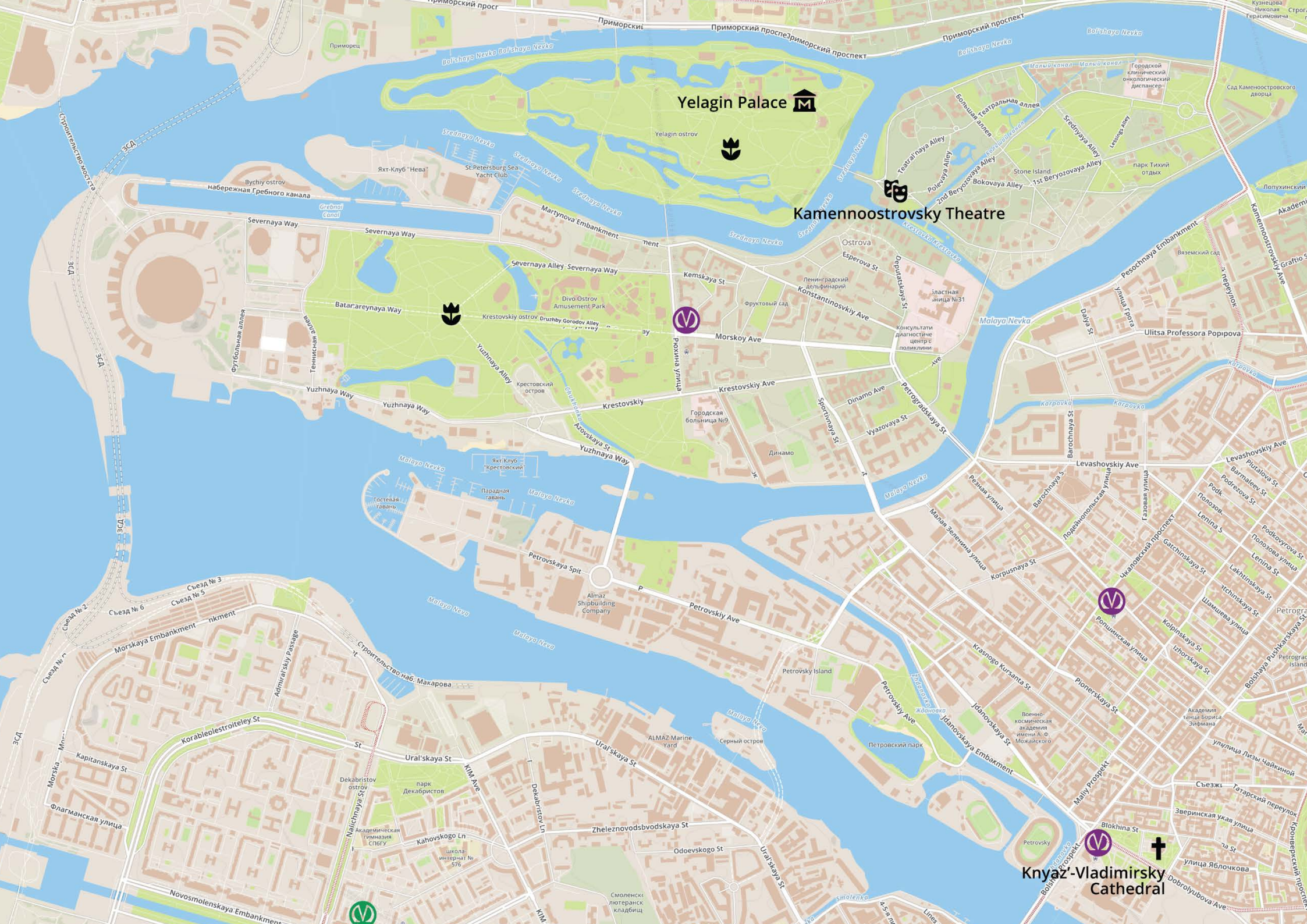
Symposium Opening Ceremony and Session 1
Sunday 19 June

Saint Petersburg Scientific Center of the Russian Academy of Sciences
Universitetskaya emb. 5

All Other Sessions
20–24 June

A.S. Popov Central Museum of Communications
Pochtamtskiy per. 4


The Spit of Vasilievsky Island
Marble Palace
Summer Gardens
Summer Gardens
Field of Mars
Birzheva
ИТМО
Old Saint Petersburg Stock Exchange
Mendeleevskaya Line
Dvortsovaya Embankment
Millionnaya St
Марсово поле
Набережная Лебяжьей канавки
Главная аллея
Лебяжья аллея
Neva
The Hermitage Theatre
Moika
St. Petersburg State University
Academy of Sciences
Palace Bridge
State Hermitage Museum
Winter Palace
St. Michael's Castle
Mikhailovsky garden
Кадетская линия В.О.
Universitetskaya Embankment
Адмиралтейская набережная
Saint Petersburg
Palace Square
Государственный Эрмитаж
Volynskiyy Ln
Большая Конюшенная улица
Savior on the Spilled Blood
Russian Museum
Russian Museum
Russian Museum of Ethnography
Румянцевский сад
Admiralty
Admiralteysky ostrov
Mikhaylovsky Theatre
Михайловский сквер
Sadovaya
The Bronze Horseman
Alexander Garden
Philharmonia
Stroganov Palace
Senatskaya Sq
Galernaya St
St. Isaac's Cathedral
St Isaac's Cathedral
Comfort Hotel
Kazansky Cathedral
Kazan Cathedral
Griboyedov Canal
Конногвардейский бульвар
Moika
Angleterre Hotel
Думская улица
Bolshoy Gostiny Dvor
Галерная улица
Yakubovicha St
VENUE
Central Museum of Communications
Kazanskiy ostrov
Казанская улица
Санкт-Петербургский государственный экономический университет
Sadovaya St
Alexandrinsky Theatre
Grivtsova Ln
переулок Антоненко
Fonarnyy Ln
Truda St
Saint Petersburg State University of Aerospace Instrumentation
Больница №28 "Максимилиановская"
СПб ГУ ИТМО
Lomonosova St
Spassky ostrov
The Central Navy Museum
Yusupov Palace
Apraksin Dvor


Yelagin Palace
Kamennoostrovsky Theatre
Knyaz'-Vladimirsky Cathedral
Приморский проспект
Приморский
Приморец
Bol'shaya Nevka
Srednaya Nevka
Malaya Nevka
Yelagin ostrov
Bychiy ostrov
Krestovskiy ostrov
Divo Ostrov Amusement Park
Ostrova
Petrovskiy Island
Almaz Shipbuilding Company
ALMAZ Marine Yard
Dekabristov ostrov
Серный остров
Петровский парк
Крестовский остров
Парадная гавань
Гостевая гавань
Яхт-Клуб "Крестовский"
St.Petersburg Sea Yacht Club
Яхт-Клуб "Нева"
Городская больница №9
Динамо
Ленинградский дельфинарий
Фруктовый сад
Консультативно-диагностический центр с поликлиникой
Городской клинический онкологический диспансер
Сад Каменноостровского дворца
парк Тихий отдых
Академия танца Бориса Эйфмана
Военно-космическая академия имени А. Ф. Можайского
Академическая гимназия СПбГУ
школа-интернат № 576
Смоленское лютеранское кладбище
Набережная Гребного канала
Grebnoi Canal
Строительство моста
ЗСД
Severnaya Way
Severnaya Alley
Martynova Embankment
Kemskaya St
Batareynaya Way
Yuzhnaya Way
Yuzhnaya Alley
Krestovskiy
Krestovskiy Ave
Morskoy Ave
Konstantinovskiy Ave
Esperova St
Deputatskaya St
Pesochnaya Embankment
Ulitsa Professora Popirova
Kamennoostrovskiy Ave
Dalya St
Petrogradskaya St
Dinamo Ave
Sportivnaya St
Vyazovaya St
Azovskaya St
Gnukhonka
Petrovskaya Spit
Petrovskiy Ave
Morskaya Embankment
Admiralskiy Passage
Строительство наб. Макарова
Съезд № 2
Съезд № 3
Съезд № 5
Съезд № 6
Съезд № г.
Korableplestroiteley St
Ural'skaya St
Kapitanskaya St
Флагманская улица
Nalichnaya St
KIM Ave
Dekabristov Ln
Zheleznovodskaya St
Kahovskogo Ln
Odoevskogo St
Novosmolenskaya Embankment
Smolenka
Barochnaya St
Barochnaya S.
Levashovskiy Ave
Pirtalova St
Barmaleev St
Podrezova St
Podk.
Полозов.
Lenina St
Podkovyrova St
Полозова улица
Lakhtinskaya St
Itchinskaya St
Шамшева улица
Kolpinskaya St
Izhorskaya St
Bolshaya Pushkarskaya St
Petrogradskaya Side
Ропшинская улица
Чкаловский проспект
Gatchinskaya St
Корпусная St
Малая Зеленина улица
Резная улица
Газовая улица
Лодейнопольская улица
Krasnogo Kursanta St
Pionerskaya St
Jdanovskaya St
Jdanovskaya Embankment
Petrovskiy Ave
Mally Prospekt
Blokhina St
Petrovsky
Улица Лизы Чайкиной
Съезжая
Татарский переулок
Зверинская улица
улица Яблочкова
Dobrolyubova Ave
Кронверкский проспект
Малый канал
Большая Театральная аллея
Teatral'naya Alley
Polevaya Alley
2nd Beryozovaya Alley
Bokovaya Alley
1st Beryozovaya Alley
Stone Island
Lesnoy Alley
Srednyaya Alley
Большая аллея
Лопухинский
Вяземский сад
Улица Грота
Kamennoostrovskiy Graftio S.
Malaya Nevka
Karpovka
Futbol'naya alleya
Tennisnaya
Petrovskiy Ave
Ulitsa Professora Popirova
Levashovskiy Ave
Barochnaya S.
Lenina S.
Podk.
Полозов.
Podkovyrova St
Полозова улица
Lakhtinskaya St
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Шамшева улица
Kolpinskaya St
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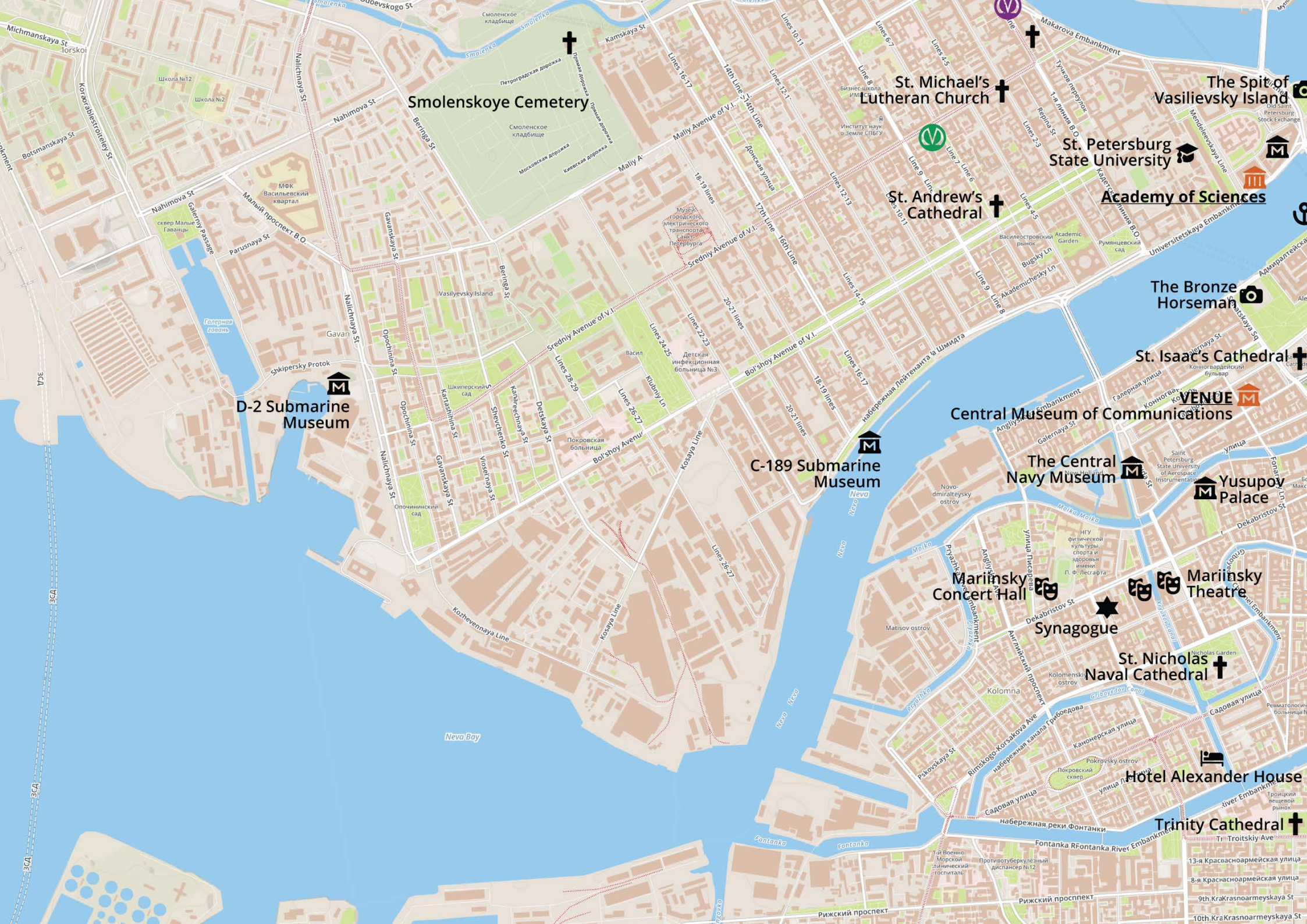
улица Яблочкова
Dobrolyubova Ave
Кронверкский проспект


Smolenskoye Cemetery
St. Michael's Lutheran Church
The Spit of Vasilievsky Island
St. Petersburg State University
Academy of Sciences
St. Andrew's Cathedral
The Bronze Horseman
St. Isaac's Cathedral
VENUE
Central Museum of Communications
D-2 Submarine Museum
C-189 Submarine Museum
The Central Navy Museum
Yusupov Palace
Mariinsky Concert Hall
Mariinsky Theatre
Synagogue
St. Nicholas Naval Cathedral
Hotel Alexander House
Trinity Cathedral
Neva Bay
Vasilyevsky Island
Kozhevennaya Line
Kosaya Line
Sredniy Avenue of V.I.
Bol'shoy Avenue of V.I.
Nalichnaya St
Gavanskaya St
Opochinina St
Shkipersky Protok
Nahimova St
Malyy Avenue of V.I.
Universitetskaya Embankment
Makarova Embankment
Dekabristov St
Fontanka
Neva
Matisov ostrov
Kolomna
Novo-dmiralteysky ostrov
Vasilevsky Island
Smolenskoye Cemetery
Смоленское кладбище
Школа №12
Школа №2
МФК Васильевский квартал
Малый проспект В.О.
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Галерный Passage
Parusnaya St
Botsmanskaya St
Korablestroiteley St
Michmanskaya St
Galernaya Harbour
Шкиперский сад
Опочининский сад
Покровская больница
Детская инфекционная больница №3
Институт наук о Земле СПБГУ
Музей городского электрического транспорта Санкт-Петербурга
Василеостровский рынок
Румянцевский сад
Academic Garden
Bugsky Ln
Akademichesky Ln
Repina St
Mendeleevskaya Line
Old Saint Petersburg Stock Exchange
Admiralteyskaya Sq
Konnogvardeysky Bulvar
Galernaya St
Galernaya ulitsa
Angliyskaya Embankment
Saint Petersburg State University of Aerospace Instrumentation
НГУ физической культуры, спорта и здоровья имени П. Ф. Лесгафта
Fonarnyy Ln
Dekabristov St
Moyka
Pryazhka
Angliyskiy prospekt
ulitsa Pisareva
Nicholas Garden
Kolomenskiy ostrov
Griboyedov Canal
Sadovaya ulitsa
Kanonerskaya ulitsa
Rimsko-Korsakova Ave
naberezhnaya kanala Griboyedova
Pskovskaya St
Pokrovsky ostrov
Pokrovskiy skver
ulitsa Pekarskaya
naberezhnaya reki Fontanki
River Embankment
Troitskiy vegetable market
Troitskiy Ave
13-ya Krasnoarmeyskaya ulitsa
8-ya Krasnoarmeyskaya ulitsa
9th Krasnoarmeyskaya St
10th Krasnoarmeyskaya St
Rizhskiy prospekt
1-y Voyenno-Morskoy klinicheskiy gospital
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Kadetskaya liniya B.O.
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14th Line
16th Line
17th Line
Line 6
Line 7
Line 8
Line 9
Donskaya ulitsa
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Kanareechnaya St
Shevchenko St
Detskaya St
Vyselnaya St
Klubniy Ln
Berynga St
Kamskaya St
Smolenka
Petrogradskaya dorozhka
Pryamaya dorozhka
Moskovskaya dorozhka
Kievskaya dorozhka
Galernyy Passage
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16th Line
17th Line
Line 6
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Line 8
Line 9
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Kartashikhina St
Kanareechnaya St
Shevchenko St
Detskaya St
Vyselnaya St
Klubniy Ln
Berynga St
Kamskaya St
Smolenka
Petrogradskaya dorozhka
Pryamaya dorozhka
Moskovskaya dorozhka
Kievskaya dorozhka
Galernyy Passage
Shkipersky Protok
Galernaya gavan'
Neva Bay
Fontanka
Neva


Komarov Botanical Institute RAS
Botanical Garden
Rosenshtein-Belogrud House
St. Petersburg Finlyandsky Railway Station
Artillery Museum
St. Petersburg Mosque
Zoo
Peter and Paul Fortress
Peter and Paul Cathedral
Smolny Cathedral
Aptekarskaya Embankment
Karpovka River Embankment
Petrovskaya Embankment
Petrogradskaya Embankment
Arsenalnaya Embankment
Smol'nayaya Embankment
Bol'shaya Nevka
Nevka
Nevo
Lesnoy prospekt
Chugunnaya ulitsa
Arsenalnaya ulitsa
Kondratyevskiy prospekt
Polyustrovskiy prospekt
Ulitsa Zhukova
Minerальная ulitsa
Boткinskaya ulitsa
Kronverkskiy prospekt
Malaya Posadskaya St
Rentgena St
Mira St
Dvinskaya ulitsa
Ulitsa Lva Tolstogo
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Symposium Programme

Time	Sunday, 19 June Venue: Saint Petersburg Scientific Center of the Russian Academy of Sciences Universitetskaya emb. 5	
14:30	Registration	
16:00	Opening ceremony	
16:30	Session 1. Plant behavior and ecology Chair: František Baluška	Dieter Volkmann, University of Bonn, Germany Pioneers in plant signaling
17:00		František Baluška, University of Bonn, Germany Root apex navigation via transition zone energides
17:35		Stefano Mancuso, Università di Firenze, Italy Universality in root behaviour
18:10		Olga Voitsekhovskaja, Komarov Botanical Institute RAS, Russia In memory of Yuri Gamalei, who studied integrative networks of plasmodesmata in relation to plant ecophysiology and adaptation.
18:30 until 21:00	Welcome reception	

Time	Monday, 20 June Incorporated sessions of School for early career and young scientists "Plant stress signaling" Venue: Hall A; A.S. Popov Central Museum of Communications, Pochtamtskiy per. 4	
09:00	Session 2. Ion channels, transporters and electrophysiology Chairs: Frans Maathuis, Vadim Demidchik	Rainer Hedrich, University of Würzburg, Germany From genome research: Venus flytrap exploits plant defenses in carnivorous lifestyle
09:50		Frans Maathuis, University of York, UK The vacuolar channel TPK1 forms a complex with a regulatory kinase involved in ABA induced stomatal closure
10:20		Igor Pottosin, Universidad de Colima, Mexico TPC1-based Ca^{2+} signaling: Slow and steady wins the race
10:50		Mary J. Beilby, University of New South Wales, Australia Modeling *Chara* action potential under salinity stress: Similarities to animal Ca^{2+} signaling?
11:10	Coffee and posters	
11:35	Session 2. Ion channels, transporters and electrophysiology Chairs: Frans Maathuis, Vadim Demidchik	Vadim Demidchik, Belarusian State University, Belarus; Komarov Botanical Institute RAS, Russia Cation channels are the target of ROS and oxidative stress in plants
12:05		Lana Shabala, University of Tasmania, Australia Membrane transporters mediating root signalling and adaptive responses to oxygen deprivation and soil flooding
12:30		Vladimir Vodeneev, University of Nizhniy Novgorod, Russia Variation potential in higher plants: Mechanisms of generation and propagation
12:50		Maria Shishova, St. Petersburg State University, Russia Ca^{2+} and H^{+} signaling in early auxin transduction

13:05		Vilma Kisnieriene, Vilnius University, Lithuania Neuroactive compounds and electrical signaling in “green axon” *Nitellopsis obtuse*
13:20	Lunch	
14:10	Session 3. Organelle, cell-to-cell and long distance signalling Chairs: Roberto Bassi Tessa Burch-Smith	Roberto Bassi, University of Verona, Italy A comparative analysis of photosynthetic light use efficiency regulation mechanisms from unicellular algae to higher plants through mosses
14:40		Ayumi Tanaka, Hokkaido University, Japan Mg-dechelatase initiates chlorophyll degradation and controls the gene expression of chlorophyll metabolism
15:10		Elena Tyutereva, Komarov Botanical Institute RAS, Russia Mechanisms of light stress tolerance in barley mutant plants lacking chlorophyll b
15:30		Natalia Pshybytko, Institute of Biophysics and Cell Engineering NAS, Belarus Role of ferredoxin redox state in chloroplasts adaptation to heat stress
15:50		Alberta Pinnola, University of Verona, Italy Binding of the second messenger Zeaxanthin upon high light stress changes the functional properties of the LHCSR1 protein from *Physcomitrella patens*
16:10	Coffee and posters	
16:35	Session 3. Organelle, cell-to-cell and long distance signalling Chairs: Roberto Bassi Tessa Burch-Smith	Christine Faulkner, JIC, UK Receptor-mediated regulation of intercellular communication during pathogen attack
17:05		Tessa Burch-Smith, University of Tennessee, USA Regulation of intercellular trafficking: the chloroplast connection
17:35		Sergey Lomin, Institute of Plant Physiology RAS, Russia Cytokinin signal transduction is obviously initiated in the endoplasmic reticulum
18:05		Andrey Solovyev, Moscow State University, Russia Plant virus movement proteins encoded by triple gene block: functions, origin and evolutionary links
18:25		Anna Komarova, Moscow State University, Russia Cyclosis-mediated long distance communications of chloroplasts
18:45	Wine and posters. All posters with a focus on Sessions 1 to 5	

Time	Tuesday, 21 June Incorporated sessions of School for early career and young scientists "Plant stress signaling" Venue: Hall A; A.S. Popov Central Museum of Communications, Pochtamtskiy per. 4	
09:00	Session 4. Stress-induced signaling Chairs: Sergey Shabala, Marc Knight	Jimmy Botella, University of Queensland, Australia Plant G proteins come of age: Breaking the signaling bond with animal models
09:30		Sergey Shabala, University of Tasmania, Australia Sensing and signalling salt stress in plants
10:00		Axel Mithöfer, Max Planck Institute for Chemical Ecology, Germany CMLs in calcium-mediated plant defense against herbivory
10:30		Tatiana Bibikova, Moscow State University, Russia Possible components of sodium sensing pathway in plants
10:50		Vladislav Yemelyanov, St. Petersburg State University, Russia The role of polyamines in signaling and adaptation to oxygen deprivation and subsequent re-aeration in plants
11:10	Coffee and posters	
11:35	Session 4. Stress-induced signaling Chairs: Sergey Shabala, Marc Knight	Marc Knight, Durham University, UK Signalling in response to changes in low temperature in *Arabidopsis*
12:05		Emilio Gutierrez-Beltran, Swedish University of Agricultural Sciences, Uppsala, Sweden Molecular composition of stress granules in *Arabidopsis*
12:30		Thomas Vincent, JIC, UK Defending plants against the World's most pesticide-resistant insect, *Myzus persicae*: A role for calcium
12:50		Gioia Lenzoni, Durham University, UK Interaction between heat and light in chloroplast calcium signaling
13:10	Lunch	
14:00	Excursion to Peterhof	
19:00 until 23:00	Conference dinner A.S. Popov Central Museum of Communications, Pochtamtskiy per. 4	

Time	Wednesday, 22 June Incorporated sessions of School for early career and young scientists "Plant stress signaling" Venue: Hall A; A.S. Popov Central Museum of Communications, Pochtamtskiy per. 4	
09:00	Session 5. Programmed cell death and autophagy Chairs: Olga Voitsekhovskaja, Diane Bassham	Diane Bassham, Iowa State University, USA RNA turnover in the plant vacuole via autophagy
09:30		Yule Liu, Tsingua University, China Cytoplastic glyceraldehyde-3-phosphate dehydrogenases interact with ATG3 to negatively regulate autophagy and immunity in plants
10:00		Andrei Vartapetian, Moscow State University, Russia Phytaspases: role in plant cell death and beyond
10:30		Farida Minibaeva, Kazan Institute of Biochemistry and Biophysics RAS, Russia Redox regulation of autophagy in plants
11:00	Coffee and posters	
11:30	Session 5. Programmed cell death and autophagy	Olga Voitsekhovskaja, Komarov Botanical Institute RAS, Russia Autophagic degradation of plant organelles

12:00	Chairs: Olga Voitsekhovskaja, Diane Bassham	Patrice Thuleau, CNRS, Toulouse, France Cytosolic glyceraldehyde-3-phosphate dehydrogenase is involved in sphingolipid signaling in plants
12:30		Viera Mackievic, Belarusian State University, Belarus Mechanisms of NaCl- and hydroxyl radical-induced programmed cell death in *Arabidopsis thaliana* roots
12:55	Lunch	
13:50	Session 6. Complex adaptive and neuron-like reactions Chairs: Stefano Mancuso, Liz Van Volkenburg	Alexander G. Volkov, Oakwood University, USA Electrical networks in plants, fruits and seeds
14:20		Liz Van Volkenburg, University of Washington, USA Plant behavior, flipped
14:50		Bernd Mueller-Roeber, University of Potsdam, Germany The control of growth plasticity by NAC transcription factors
15:15		Salma Balazadeh, University of Potsdam, Germany A novel control module for thermomemory in plants
15:35		Andrej Pavlovič, University of Olomouc, Czech Republic Short- and long-distance electrical signaling in carnivorous plants
15:55	Coffee and posters	
16:15	Session 6. Complex adaptive and neuron-like reactions Chairs: Stefano Mancuso, Liz Van Volkenburg	Susan Murch, University of British Columbia, Canada Auxins and indoleamines: Signaling in plant regeneration
16:45		Yariv Brotman, Ben Gurion University of the Negev, Israel Exploring the metabolome of higher plant species
17:15		Mannie Liscum, University of Missouri, USA Phototropin responses: Getting from intracellular biology to organ-level behavior, adaptation and fitness
17:40		Greg B. Clark, University of Texas, USA Using a self-referencing biosensor and kinematics to assay physiological differences mediated by altered apyrase expression in transgenic roots
18:00		Paco Calvo, University of Murcia, Spain Guidance of circumnutation of climbing bean stems: An ecological exploration
18:20 until 21:00	Wine and posters. All posters with a focus on Sessions 6 to 10	
Satellite Session "Techniques in Imaging and Photosynthesis Research" Venue: Hall B; A.S. Popov Central Museum of Communications, Pochtamtskiy per. 4		
16:30	Maria Lemak, Nikon Instruments Nikon confocal systems: what's new in advanced fluorescent microscopy	
16:45	Rick L. Garcia, LI-COR Biosciences (USA). Exploring the power of parallel measurements of electron transport, CO_2 and H_2O flux in plant leaves	
17:00 until 19:00	WORKSHOP (LI-COR, LABINSTRUMENTS). Li-Cor and LabInstrumens organize a workshop on new systems to study photosynthesis in vivo and will introduce the newest LI-6800 Portable Photosynthesis System (Li-Cor).	

Time	Thursday, 23 June Incorporated sessions of School for early career and young scientists "Plant stress signaling" Venue: Hall A; A.S. Popov Central Museum of Communications, Pochtamtskiy per. 4	
9:00	Session 7. Phytohormone signaling Chairs: Georgy Romanov, Kirill Demchenko	Georgy Romanov, Institute of Plant Physiology RAS, Russia Cytokinin signaling system: new developments
9:25		Kirill Demchenko, Komarov Botanical Institute RAS, Russia Early cellular events and auxin response during lateral root initiation in the primary root meristem of squash
9:50		Joseph Dubrovsky, Institute of Biotechnology, Mexico Root developmental plasticity: to maintain the root apical meristem or become determinate?
10:15		Viktor Ivanov, Institute of Plant Physiology RAS, Russia Cytokinins determine *Arabidopsis* root meristem size and root growth rate by controlling cell proliferation rather than cell differentation
10:35		Juha Immanen, University of Helsinki, Finland Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity
10:55		Sebastjen Schoenaers, University of Antwerpen, Belgium An ARF7/ARF19-regulated kinase controls calcium-dependent tip growth in *Arabidopsis*
11:15	Coffee and posters	
11:40	Session 8. Interaction with bacteria and fungi Chairs: Igor A. Tikhonovich, Katharina Pawlowski	Igor A. Tikhonovich, ARRIAM, Saint Petersburg, Russia Plant-microbe signal exchange leads to the highly specific symbiosis development
12:10		Katharina Pawlowski, Stockholm University, Sweden Evolution of actinorhizal symbioses: Root nodules induced by members of the basal group of *Frankia* strains
12:35		Ingo Dreyer, University of Talca, Chile Cooperation through competition: Dynamics and microeconomics of a nutrient trade system in arbuscular mycorrhizal symbiosis
13:00		Ton Timmers, CRNS, Toulouse, France Nod factor perception and signal transduction during endosymbiotic interactions of *Medicago truncatula*
13:25		Ralf Oelmüller, University of Jena, Germany Local and systemic signaling in plant/microbe interaction mediated by a novel chemical mediator
13:45	Late Lunch	
14:35	Session 9. Response to radiation and gravity Chairs: Sergey Medvedev, Galina Shevchenko	Galina Shevchenko, Kholodny Insitute of Botany NAS, Ukraine Genome stability in plants from Chernobyl zone is facilitated by DNA-repair pathways
15:00		Tatiana Gorshkova, Kazan Institute of Biochemistry and Biophysics RAS, Russia Unexpected turns in gravitropic curvatures
15:25		Namik Rashydov, Institute of Cell Biology and Genetic Engineering NAS, Ukraine Long remote carry out of light and gene activity signals in plant seedling
15:45		Tatiana Bilova, St. Petersburg State University, Russia Simulated microgravity induces specific alterations in the metabolome and proteome of germinated *Brassica oleracea* and *Brassica napus* seeds

16:05		Marcela Hola, Czech Academy of Sciences, Prague, Czech Republic Molecular basis of environmental stresses-induced mutagenesis in *Physcomitrella patens*
16:25		Miroslav Perniš, Institute of Plant Genetics and Biotechnology, Slovakia Closer look on Chernobyl area-grown soybean seeds and their adaptation to increased level of ionizing radiation
16:45		Gregory A. Pozhvanov, St. Petersburg State University, Russia Ethylene is involved in the actin cytoskeleton rearrangement during the root gravitropic response of *Arabidopsis thaliana*
17:05	Coffee and posters	
17:25	Session 10. ROS/RNS and neurotransmitter signaling Chairs: Boris Ivanov, Satish Bhatla	Boris N. Ivanov, Institute of Basic Biological Problems RAS, Russia Formation of reduced reactive oxygen species in a photosynthetic electron transport chain, and their signaling role
17:50		Satish Bhatla, Delhi University, India Nitric oxide and melatonin crosstalk with reactive oxygen species scavenging enzymes in modulating abiotic stress tolerance
18:15		Viktor Tsyganov, ARRIAM, Saint Petersburg, Russia Pea (*Pisum sativum* L.) nodule development: Reactive oxygen species, antioxidants and phytohormones
18:35		Yana Toporkova, Kazan Institute of Biochemistry and Biophysics RAS, Russia Features of lipoxygenase cascade of different plant species
18:55	Closing remarks	

Time	**Friday, 24 June** **Discussion Groups within School for early career and young scientists "Plant stress signaling"** Venue: Halls A and B; A.S. Popov Central Museum of Communications, Pochtamtskiy per. 4		
9:00-11:00	Discussion Group A: **Neuron-like aspects of plant adaptive behavior** 15 participants 3-min presentations	Discussion Group B: **Stress: survival or death?** 15 participants 3-min presentations	Discussion Group C: **Encoding stress specificity** 15 participants 3-min presentations
11:00	Transportation to Sessions and Early Lunch		
12:30-16:00	Workshop "Cell biology techniques in plant signaling studies"	Introduction Leaders: Kirill N. Demchenko, Alexandra N. Ivanova, Grigory A. Pozhvanov St. Petersburg State University, Universitetskaya nab. 7/9	
		Session 1. St. Petersburg State University, Research Park Demonstrations based on Leica Microsystems CLSM Facilities Universitetskaya nab. 7/9	
		Session 2. Komarov Botanical Institute RAS, The OPTEC confocal microscopy reference Center in the Core Facilities "Cell and Molecular Technologies in Plant Science" in the Komarov Botanical Institute RAS (St.-Petersburg, Russia)	

Scientific Organising Committee

Co-heads

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Vadim Demidchik, Belarusian State University, Belarus; Komarov Botanical Institute RAS, Russia

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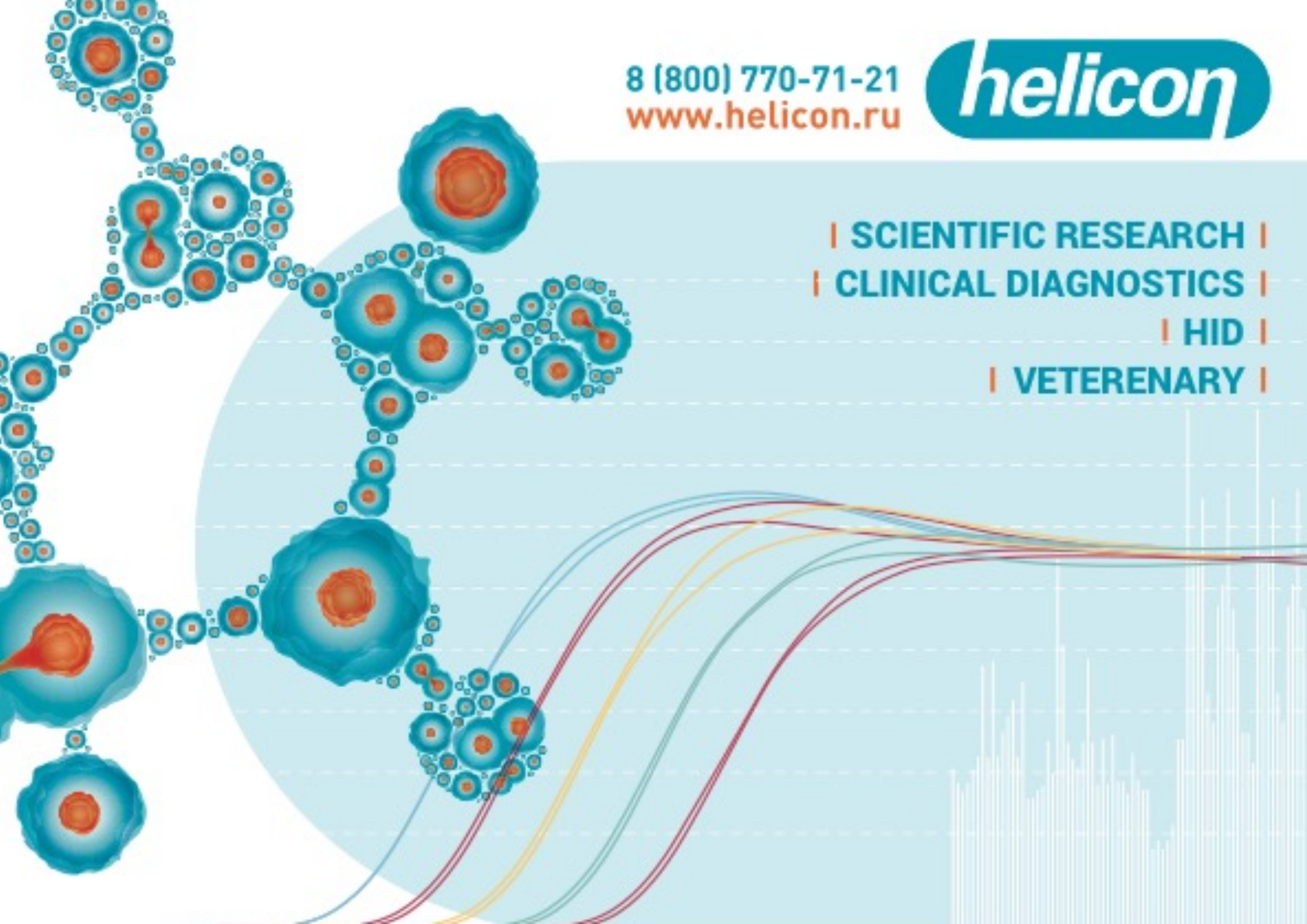

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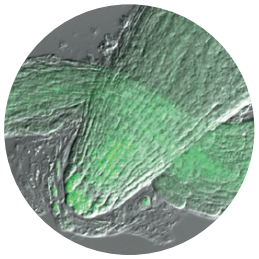

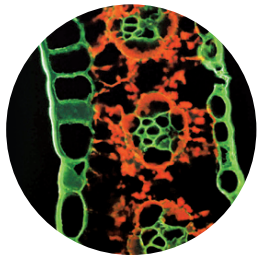

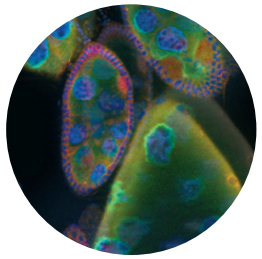

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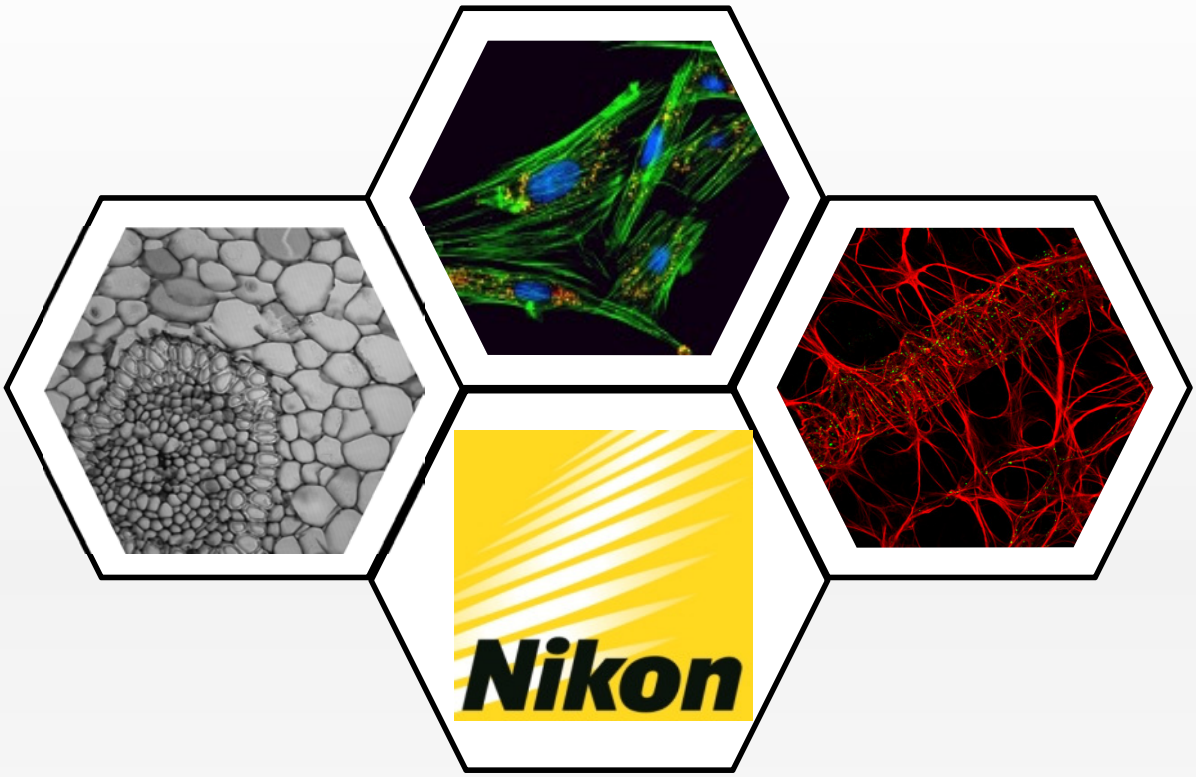

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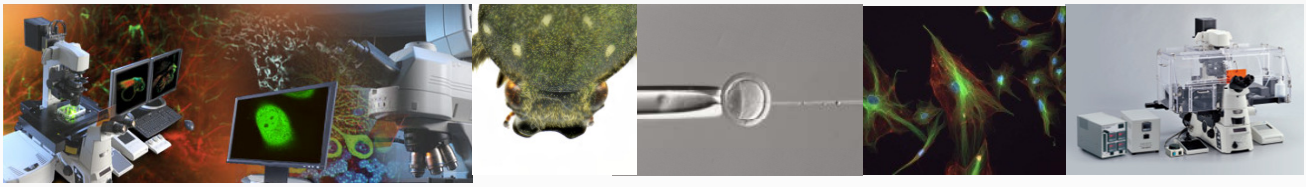

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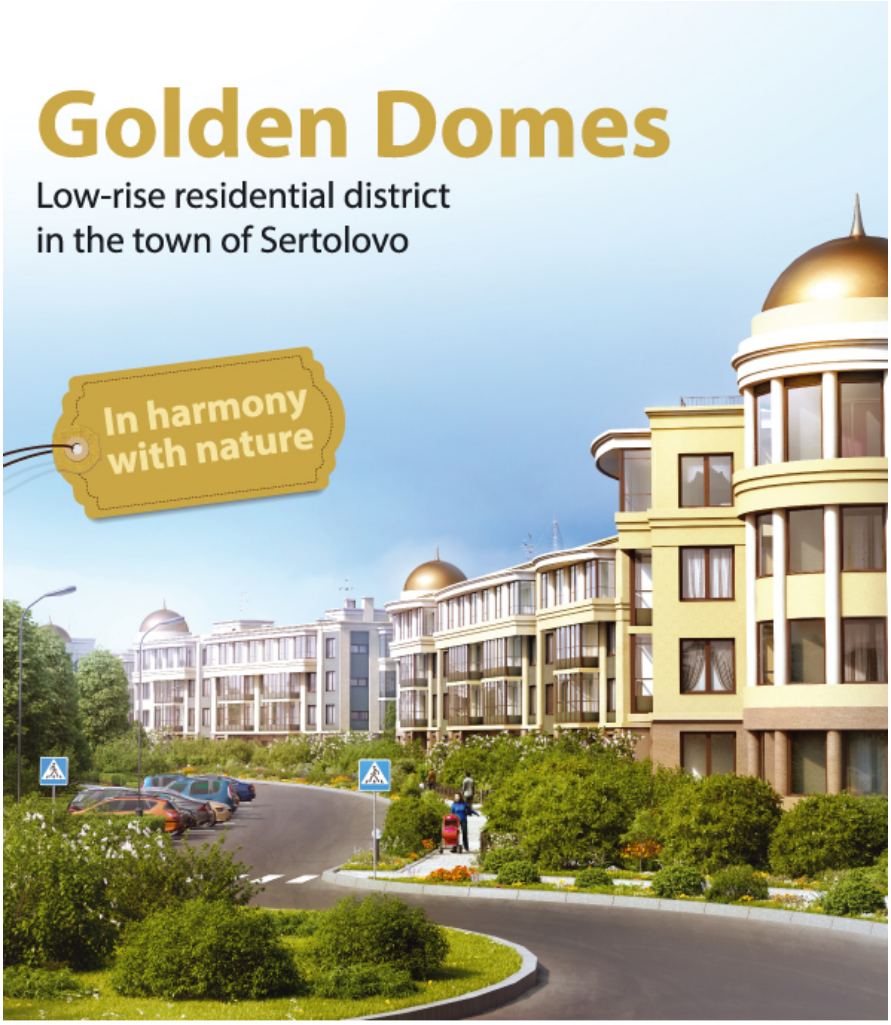

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Scientific Sessions

SESSION 1

Plant Behavior and Ecology

The effect of different concentrations of hypericin hormone stimulates the production of the plant Hypericum

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Naphtodianthrone is a light-sensitive hypericin photodynamic activaity. Hypericum extracts and compounds from the plant is useful in the treatment of many diseases. The research was conducted in the Laboratory of Biotechnology and Plant Breeding of Islamic Azad University. In this study, the effect of different concentrations of hormones 2.4-D+BAP and NAA+KIN in basal medium (MS) to stimulate the production of hypericin was investigated. Tiny samples of the leaves and stems of sterile plants cultivated in basal medium (MS) has been prepared and the MS medium containing 2.4-D+BAP and NAA+KIN hormone concentrations (control, 0/5 and 1 mg l) were transported at a temperature of 21 ± 3 °C in 16 hours of light and 8 hours of darkness and light conditions were maintained. Methanol extracts of this plant by HPLC analyzes to examine changes in hypericin. The results showed that hypericin range of chromatogram standard range of hypericin in 05:38 minutes also show the whole standard of hypericin. So the percentage of hypericin in the control sample of 1% is at 06:02. Different concentrations hormones 2.4-D+BAP and NAA+KIN reflects the impact on stimulating the production of hypericin in the plant is not actually had any effect on stimulating the production of hypericin in vitro. Therefore it is recommended that hormones or other growth regulators can be used to stimulate the production of hypericin.

Root Apex Navigation via Transition Zone Energides

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Roots explore complex environment of soil. In this very challenging endeavour, root apices traverse enormous distances – searching for water and nutrients; but avoiding toxic, dry and dangerous zones. Any wrong turnings are energetically expensive. Root apex transition zone integrates diverse inputs from endogenous (hormonal) and exogenous (sensorial) stimuli and translates them into signalling and motoric outputs as adaptive differential growth responses. These underlie the root tropisms and other aspects of adaptive root behaviour. The oscillatory nature of the transition zone is in accordance with the proposal of Charles and Francis Darwin that this root apex zone may be considered to be a plant-specific 'brain-like' organ endowed with a sensitivity which controls its navigation through soil. Recently, a revision of the cell theory has been proposed, which has several implications for both cell physiology and neurobiology. This revision is founded on adapting the old Julius von Sach's proposal of the Energide as the basic unit of eukaryotic life. Sachs proposed the Energide concept by postulating that the nucleus and its protoplasm represent the smallest unit of eukaryotic life, while the cell periphery is a secondary structure generated by the active Energide for its shelter and protection. The Energide concept is central for the information handling in the central nervous system. Here, the Energide paradigm is discussed from the root apex navigation perspective, suggesting also a revision of the Darwinian concept of the root apex 'brain-like' organ in the root apex navigation.
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Plant response to a new irradiance level depends on the process of nitrate assimilation

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In plants, the ratio of carbon to nitrogen (C/N balance) is important for regulation of different processes, including photosynthesis, secondary metabolite synthesis and defense responses, nitrogen assimilation, flowering and germination. Despite progress in studying of some aspects of this regulation its mechanism remains elusive. Because both low light and high nitrogen nutrition must decrease the C/N balance we compared the influence of abrupt changes in irradiance and N nutrition on photosynthesis and photosynthetic carbon metabolism.

The reaction of two plant species C-3 plant sunflower (*Helianthus annuus* L.) and C-4 plant maize (*Zea mays* L.) to changes in irradiance and nitrate nutrition level was studied. Plants were grown under natural conditions. In 5 days prior the experiment some plants were transferred to lower irradiance (50% of full sunlight). On the eve of experiment some plants were watered with KNO3 solution. In 3 min prior to 2 min exposure to $^{14}CO_2$ a group of shaded plants was shifted to full sunlight, and some full lit plants were transferred to 50% lower irradiance and then a fully grown leaf of every plant was exposed to $^{14}CO_2$ under current lighting conditions.

$^{14}CO_2$ assimilation by all shaded plants was lower than that by full lit plants. However, sunflower and maize responded differently to abrupt changes in irradiance. In maize, after 3 min shading $^{14}CO_2$ assimilation was significantly lower than that of shade adapted control, and 3 min of full sunlight was enough to restore the level of CO_2 assimilation by shade adapted plants to almost the level of sun adapted plants. The negative effect of sudden decrease in irradiance was especially prominent on the background of high nitrate nutrition. Oppositely, after short-term reduction in irradiance sunflower plants decreased $^{14}CO_2$ assimilation only to the level of shaded control, but shade adapted plants were unable to restore the level of $^{14}CO_2$ assimilation to the level of sun adapted control after 3 min of full sunlight exposure. Nitrate nutrition somewhat increased the rate of $^{14}CO_2$ assimilation by sunflower under all lighting regimes, except for under abrupt transferring from shade to light.

A usual metabolic response to increased N was observed in both plants species - nitrate increased ^{14}C distribution to malate and amino acids. In maize, aspartate synthesis responded most prominently to changes in nitrate and irradiance level. There were not any significant changes in leaf ultrastructure under varied light regimes.

The differences observed between sunflower and maize could not be explained by their belonging to C-3 and C-4 plant types because in our earlier experiments C-3 potato plants responded similarly to C-4 maize. We suggest that the effects were related to the place of nitrate reduction. Sunflower plants are known to reduce nitrate preferentially in roots and to transport N to shoots as amino acids, while maize and potato reduce nitrate in leaves. After abrupt decrease of irradiance the ratio of ^{14}C-amino acids to ^{14}C-sugars increased significantly in maize and potato but not so prominently in sunflower. We suggest that sunflower plants quickly acclimate to low irradiance but N used in nitrogen metabolism after light decrease is partly derived from photosynthetic enzymes. That is why, in sunflower, nitrate nutrition has no negative influence on CO_2 assimilation rate under low irradiance, but photosynthesis cannot be quickly elevated after a sudden increase in irradiance. Such plants as maize and potato continue to reduce nitrate in leaves even under low light, which interferes with carbon reduction, but enables plants to keep the level of photosynthetic enzymes and to rapidly increase photosynthesis after light increase.

Thus, despite decreased nitrogen assimilation (in absolute amounts) under low light regime the flow of photoassimilates through nitrogen metabolism increases in comparison to that through carbon metabolism, indicating a lower C/N balance and resembling the situation observed under high N nutrition. Some effects observed can be associated with inhibition of photoassimilate export from leaves by nitrate.

Photosynthesizing on metal excess: Copper differently induced changes in various photosynthetic parameters in copper tolerant and sensitive *Silene paradoxa* L. populations

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This work investigated Cu-induced changes in photosynthetic activity in contrasting populations of *Silene paradoxa* L. A metallicolous Cu-tolerant population and a non-metallicolous sensitive population were grown in hydroponics and exposed to different $CuSO_4$ treatments for different times. Copper accumulation, MDA concentrations, and several photosynthetic parameters were measured to assess different effects of Cu exposure on plants from the two populations. A more efficient ability to photosynthesize in the presence of Cu excess was showed by the Cu-tolerant population with respect to the sensitive one. Interestingly, Cu-imposed limitations were present not only at a different degree, but also of different nature in the two populations. In the tolerant population, the most limiting factor to photosynthesis seemed to be Cu-imposed stomatal closure, whereas Cu-mediated biochemical limitation was scarce and Cu-mediated reduction in mesophyll conductance almost non-existent. In the sensitive population, Cu largely affected all the measured parameters, so that its photosynthetic activity experienced any kind of limitation, diffusional and especially biochemical. The lower Cu concentrations accumulated in the tolerant plant could be one of the factors concurring to the reported differences in photosynthetic activity, but also a higher capacity of internal detoxification and compartmentalization of the metal could not be excluded.

Assisted phytoremediation with castasterone and citric acid improves cadmium stress tolerance in *B. juncea* plants

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Heavy metal contamination of soils and water is a grave environmental problem. Cadmium toxicity is a serious agricultural threat. Cadmium in plants brings biochemical/ physiological/ molecular consequences. It also interferes with uptake and transport of macro/micro molecules. Thus effective remediation measures are needed to minimize heavy metal/ cadmium contamination. Various physical and chemical methods are available for the remediation of cadmium contamination but practical application of them is limited due to high cost and irreversible changes in physico-chemical and biological soil properties etc. Phytoremediation is a cost-effective, eco-friendly, in-situ applicable technique that uses green plants for cleaning and/or rendering environmental contaminants less harmful. However, phytoremediation of heavy metals is limited by low bioavailability of metal ions in the soil, and slow growth rate and biomass production due to the toxic effects of metal ions. In contrast to continuous phytoextraction which relies on natural metal hyperaccumulators, assisted phytoextraction involves tolerant plant species and supplementary agents to increase the contaminant bioavailability and accumulation. To increase available fraction of soluble metal ions in the soil, various organic and inorganic amendments in the form of chelating agents are used. Chelating agents assisted phytoremediation is considered as a viable strategy for phytoremediation of heavy metals. Phytoextraction aided by phytohormones treatment is an emerging concept in plant growth regulator (PGR) assisted phytoremediation. Plant hormones in context to heavy metal phytoremediation have potential to increase plant growth, stress tolerance and metallic accumulation. The use of plant growth promoting substances improves phytoextraction by increasing shoot and root growth and the biomass yield and enhances the effectiveness of antioxidant system in plants thereby facilitating greater accusation and accumulation of trace elements. Moreover, the chelate and PGRs assisted methods are often combined and used together to enhance phytoextraction efficiency. BRs have ability to enhance resistance potential of plant against excess heavy metal by boosting antioxidant defense system and net photosynthetic rate. Castasterone (CS) is a biologically active, 6-oxo type brassinosteroid. Present study was therefore planned to study that castasterone application can improve potential of citric acid (CA) assisted phytoremediation of cadmium by alleviating cadmium induced oxidative stress and inhibition in photosynthetic parameters in 60 days old *B. juncea* plants grown in cadmium spiked soil. It was

observed Castasterone and/or citric acid application lowered MDA, H_2O_2 content and superoxide anion production rate and enhance net photosynthetic rate, transpiration rate and activities of antioxidative enzymes (SOD, POD and CAT) and metal accumulation. This is the first study which supports that CS and CA application can improve cadmium phytoextraction in *B. juncea* by amelioration of cadmium toxicity.

Silicon role in plant defense against heavy metal stress

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Plants have involved different mechanisms to minimize the damage caused by high concentrations of heavy metals. First, many plants exposed to toxic concentrations of metals attempt to prevent or reduce uptake into root cells by restricting metal ions to the apoplast, binding them, or by inhibiting long distance transport. Then plants activate oxidative stress defense mechanisms and the synthesis of stress-related proteins and signaling molecules. As evident from scientific data, plants exposed to HM pollution enhance the uptake of monosilicic acid and Si transport from leaves to roots. The reasons of this phenomenon and the role of silicon compounds in plant defense against heavy metal pollution are investigated very poorly.

Special methodology for determining the soluble forms of elements in the apoplast and symplast of plants has been elaborated. The investigation was conducted with rice being grown in nutrient solution which contained monosilicic acid at concentrations of 0 and 2 mMol as a source of plant-available Si. On the third week of plant growth, cadmium was added to the nutrient solution at the concentrations of 0,05; 0,50; and 1,00 mMol. The dynamic of soluble forms of Si and Cd was analyzed in the nutrient solution as well as in the apoplast and symplast of roots, stems, and leaves during the next 6 days. The obtained data has shown that low concentration of Cd in the nutrient solution didn't initiate the additional adsorption of Si by plants, but initiated the reduction in the root-to-leaf Si apoplastic transport, what is probably related to the Si participation in the Cd precipitation in the roots. At the Cd concentrations of 0, 50 and 1,00 mMol, the additional Si adsorption from nutrient solution and the reverse Si transport from leaves to roots were observed. Thus, increased concentration of Cd resulted in special mechanisms to be involved as a response to heavy metal stress. It is important that increased Cd in the root apoplast didn't lead to similar increase in the root symplast, while the Cd content in the symplast of stems and leaves increased with increasing the Cd concentration in the nutrient solution. The root cells of rice possibly have specific protective mechanism against heavy metal contamination, while the stem and leaf cells don't have such mechanism. Further investigations are necessary to elucidate the mechanisms of Si action in plant protection against abiotic stresses.

This project was supported by Hunan Administration of Foreign Experts Affairs(letter fromHunan Provincial Department of Human Resources and Social Security Functions [2015]180), China.

Population-based interactions among roots as a mechanism for intraspecific diversity-ecosystem function relationships

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Species diversity can have strong positive effects on ecosystem function, with species-rich communities yielding on average ≈70% more than monocultures. Studies of such diversity-dependent "overyielding" have focused primarily on the diversity of species, but a large and poorly understood extent of Earth's biological diversity is *within* species. Most species vary genetically within populations and across regional distributions, and many natural populations consist of functionally distinct, locally adapted "ecotypes". Functional variation within a species appears to be high enough to have significant effects on a variety of ecosystem processes, including productivity. Consistent with this, we found that increasing population/ecotype diversity of a western bunchgrass, *Pseudoroegneria spicata*, increased local aboveground productivity to a similar degree as that reported for species diversity. Ecotypes that produced larger plants overyielded at low diversity at the expense of ecotypes that produced smaller plants, and small ecotypes overyielded through complementarity at all levels of diversity. These results suggest that mass-based competition or other cryptic ecotype-specific processes had complex but

important effects on overyielding. Therefore we explored the potential for identity recognition, or signaling, among the roots of different *Pseudoroegneria* ecotypes to contribute to overyielding in plots with high intraspecific richness of this species relative to monocultures. We found that when plants from different populations were planted together in pots the total biomass yield was 30% more than in pots with two plants from the same population. Second, we found that the elongation rates of roots of *Pseudoroegneria* plants decreased more after contact with roots from another plant from the same population than after contact with roots from a plant from a different population. These results suggest the possibility of some form of detection and avoidance mechanism among more closely related *Pseudoroegneria* plants. If decreased growth after contact results in reduced root overlap, and reduced root overlap corresponds with reduced growth and productivity, then variation in detection and avoidance, perhaps a form of signaling, among related and unrelated accessions may contribute to how ecotypic diversity in *Pseudoroegneria* increases productivity.

The role of abscisic acid in wheat plants adaptation to low temperatures

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Abscisic acid (ABA) is a stress hormone that plays an important role in plants tolerance to various unfavorable factors participating in regulation of some stress-related genes expression, as well as leading to adaptive physiological and biochemical changes. Thus, the aim of our research was to investigate the effect of exogenous ABA on wheat plants response to low temperature.
Experiments were conducted with seedlings of winter wheat (*Triticum aestivum* L.) cv. Moskovskaya 39, which were exposed to temperature 4°C for 7 days. Some seedlings were placed on ABA solution (0.1 mM) one day before cold hardening. Further, the expression of the transcription factors (*WRKY*, *CBF4*, *MYB80*, *bZIP*) and *COR*-genes (*WRAB17*, *WRAB19*, *WCOR15*, *WCS120)* encoding proteins of cold response was studied. Also the dynamic of seedlings cold tolerance, the chloroplasts ultrastructure and the content of free proline were analyzed.
It was shown that the development of wheat cold tolerance was related to the increase in the expression of the transcription factors *WRKY*, *CBF4*, *MYB80*, *bZIP*, and the ABA-dependent (*WRAB17*, *WRAB19*) and ABA-independent (*WCOR15* and *WCS120*) *COR*-genes.
The changes in the dynamic of *COR*-genes expression were found at short-term (minutes, hours) effect of temperature 4°C as well as at long-term exposures. In particular it was found that the increase of wheat plants tolerance at the initial stage of cold hardening is related to the *WCOR15*, *WRAB17*, *WRAB19* and *WCS120* genes expression, while at more prolonged exposure it was observed the *WCOR15* and *WRAB17* genes expression. Exogenous ABA caused the additional increase of wheat plants tolerance during cold adaptation and upregulated the *WCOR15*, *WRAB17*, *WRAB19* genes expression both at cold-hardening (4°C) and normal (22°C) temperatures.
It should be noted that exogenous ABA induced certain changes in the chloroplasts structure: increase in their size, density of chloroplasts stroma and the formation of protrusions. Furthermore, significant raise of free proline content was observed in seedlings leaves in the presence of ABA.
Thus, the obtained data suggest an important role of ABA in wheat plants adaptation to low temperature. ABA induces changes in the genes expression and structural and functional changes (in particular, in the photosynthetic apparatus), that is a necessary component of the plants cold adaptation process.

Fatty acid composition of lipids from neeedles and chlorophyll content under vegetation conditions in Pinus sylvestris in the Baikal region

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One of the most important physiological and biochemical processes occurring in a plant organism is the photosynthesis. The changes in the content of pigments that participate in the photosynthesis process indicate the adaptive character of the reactions of a plant organism to the stress impact of the environment. It is known that the dynamics of chlorophyll and the structure of chloroplast membranes

during the vegetation and in the yearly cycle are regulated by the insolation level and the temperature conditions. An important role in the adaptation processes of plants to the environmental factors is played by fatty acids, which provide the plasticity and the integrity of photosynthetic and cell membranes. The aim of this work is to study the chlorophyll content and the fatty acid composition of lipids from needles of *Pinus sylvestris* L. under the vegetation conditions. The results of the study prove that the pigment pool of needles of Scots pine under the conditions of the Baikal region possesses high plasticity, which determines high adaptive properties of the photosynthetic apparatus to the growing conditions. During the vegetation periods with a high insolation level at different temperature and humidity conditions, the main changes affect the content of chlorophyll *b* while the dynamics of chlorophyll *a* remains almost unchanged. It was first shown that the dynamics of the chlorophyll content in LHC and the content of unsaturated fatty acids (UFA) of lipids in needles during the vegetation can have up to three peaks depending on the stress impact of the environmental factors. The spring peak of the chlorophyll content in light-harvesting complexes of photosynthetic units (LHC PSU) corresponds to the increase in the UFA content of lipids in needles, the beginning of the photosynthetic activity, and low air and soil temperatures. The spring peak of the chlorophyll content in LHC is most probably related to the protection of the photosynthetic apparatus from the negative action of low temperatures. It is assumed that there is a relation between the increase in the chlorophyll content in LHC, low water content in needles, and the increase in the UFA content in lipids, which increase the fluidity of chloroplast membranes and, thus, contribute to the protection from the damaging effect of the sunlight. The summer peak of the chlorophyll content in LHC is, probably, related to the PSU rearrangement because of the chlorophyll formation under the conditions of the temperature optimum, which is proved by the simultaneous increase in both the total chlorophyll *a, b* content and the chlorophyll content in LHC. The increase in the UFA content in lipids of needles coincides with the highest content of chlorophylls in needles in this period. It is assumed that, in the middle of summer (in July), UFA participate in the incorporation of newly formed chlorophylls into thylakoid membranes of chloroplasts and participate in the activation of the outflow of assimilates from needles to the reproductive, growing, and storage tissues. The autumn peak of the chlorophyll content in LHC, probably, indicates the preparation of the photosynthetic apparatus for winter and/or the increase in the light-harvesting function because of the two- or three-fold decrease in the illuminance level along with the active photosynthesis. The autumn (September) peak of UFA in lipids of needles corresponds to the decrease in the chlorophyll level in needles and the increase in the LHC fraction as a response to the decrease in the illuminance and the air temperature in autumn.
The dynamics of the UFA content in lipids of needles has a similar character with the indicators of the water status of needles (free and total water fractions) in the spring and summer periods. It is concluded that the interdependent dynamics of the components of the assimilation apparatus (chlorophylls and fatty acids) is a manifestation of an adaptive mechanism that provides the stability and high biological productivity of coniferous stands under the conditions of the Baikal region.
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What would change today Aristotle in his theory of the vegetative *psyche*?

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Aristotle was primarily a biologist. Searching for answers (eg. by repeating the experiences of Hippocrates) to the question about the cause of development of the organism, he created a theory of internal integrating factor and called it *psyche*. Aristotle's thinking was based on *epagoge*, some kind of intellectual induction. In XX century, the concept of the *soul* (*psyche*, *anima*) disappeared - at least in the natural sciences. Only a small group of scientists used this term explicitly referring to Aristotle. One of them was embryologist Hans Driesch (1867- 1941). He was looking for - like Aristotle - the factor responsible for the harmonized development of the organism. He concluded that it must be a non-physical factor acting within the material structures of the body (*actio immanens*). Of course, Driesch was far from understanding the existence of this intangible factor on the Descartes way (who was talking about independent existence *res cogitans* and *res extensa*; in the opinion of Descartes plants and animals are type of machines). A living creature was for Driesch - like for Aristotle - an indivisible whole - *individuum*. The question of how non-physical (*psyche*) interacts with the material structures is still - in philosophy - the subject of discussion.
1. Seeing the difference between plants and animals Aristotle talked about a *vegetative soul* (*anima vegetative, psyche threptike*) and a *sensitive soul* (*anima sensitive, psyche epithymetikon*). If Aristotle knew the results of modern biology, he probably would recognize that plants and bacteria (the existence of which he had no idea) are aware of the environment and of the some structure of their body (which

allows them to manipulate these structures and, through this acting, manipulate of objects within their environment). The plants have, therefore, a kind of "sensual life," which Aristotle attributed solely to animals and humans. Exploring (orientation) belongs to the very essence of life - is a substantial act (not an attribute, not accidental act).
2. Another issue concerning the theory of Aristotle is the question about the possibility of the existence of the *psyche* when is not possible to build its material body (we can call this the situation death or waiting). In the tradition of thinkers analyzing the theory of Aristotle the *psyche* of plants cannot be self-existent primarily due to the lack of intelligence which is the basis of self-awareness of the organism. Only the soul of a human being (*rational soul*) can exist without the body. Today, introducing some distinctions (eg. between *intelligence* and *intellectuality*), we can rethink this issue. We can say that everything that is alive - cognizes and has some kind of intelligence.
Biologists always perceived the necessity of integrating principium of living organism. When the structure of the DNA molecule was discovered the secret of life seemed to be solved. Nowadays when we see – better and better - the complex (and perfectness) of development of organisms, their orientation and manipulation, the theory of Aristotle, regardless of acceptance or non-acceptance of these two modifications, appears still inspiring.

Effect of the Ser/Ala substitution in the N-terminal extension of rice phyA expressed in *Arabidopsis* on the state of its native pools

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Phytochrome A (phyA) mediates very low and low fluence responses (VLFR and LFR) and high irradience reactions (HIR). This versatility may be connected with the existence of the two phyA native types – phyA' and phyA'' (Sineshchekov, 2010). They differ by the character of post-translational modification of the molecule at its N-terminal extension (NTE) (possibly, by the state of phosphorylation with phyA' being phosphorylated and phyA'' dephosphorylated species). In this work, we went on investigating the role of phosphorylation in the phyA differentiation and its functional implications turning to transgenic *Arabidopsis* (deficient in phyA or both phyA and phyB) over-expressing wild-type rice phyA (phyA WT) or mutant rice phyA (phyA SA) with the substitution of the first 10 serines by alanines preventing phyA phosphorylation at this site. In these transgenic lines, phyA WT led to hypersensitive VLFR and LFR responses, whereas phyA SA showed stronger HIR responses suggesting a role for phosphorylation at these serines in discriminating between the different phyA-dependent responses (Kneissl et al., 2008). Etiolated seedlings of these lines taken after 4 days of imbibition at 4 ^{0}C, then 15 min or 3 h white light (WL) germination-inducing pretreatment and 4 days of germination at 23 ^{0}C were employed in the experiments. The following parameters of the pigment in its Pr form were evaluated with the use of low temperature (85 K) fluorescence spectroscopy and photochemistry: spectral characteristics, total content [P_{tot}] and extent of the Pr→lumi-R conversion at 85 K (γ_1) allowing determination of the proportion and content of the two phyA pools (photochemically active at 85K phyA' and inactive phyA''). Fluorescence emission spectra with the maximum at 680-682 nm and half-band width of 25-30 nm were essentially the same for phytochrome in all the plant lines. [P_{tot}] of exogenous rice phyA WT and phyA SA expressed in *phyAphyB Arabidopsis* were comparable with [P_{tot}] in the wild-type *Arabidopsis* (LER) plants (0.51-0.54 r.u. vs. 0.48 r.u.). However, in the presence of endogenous *Arabidopsis* phyB (*phyA* background) the content of rice phyA WT and phyA SA was lower by approx. 20% than in LER (0.38-0.4 r.u. vs 0.48 r.u.) suggesting that phyB may inhibit the synthesis of the exogenous rice phyA. Proportion of the pools of wild-type phyA (phyA'/phyA'') varied depending on the duration of germination-inducing white light seed pretreatment: for endogenous phyA in LER from 45/55% after 15 min WL to 56/44% after 3 h WL, and for rice phyA WT in *phyA Arabidopsis,* respectively, from 39/61% to 68/32%. Similar effect of germination-inducing preillumination was shown earlier for *Adiantum* phy1 expressed in *Arabidopsis* (Sineshchekov et al., 2014). In *phyAphyB Arabidopsis*, phyA'/phyA'' after 3 h WL was 58/42% – close to that in LER and lower than in *phyA Arabidopsis*. The latter suggests that the balance of the pools in phyA WT is also affected by the presence of endogenous *Arabidopsis* phyB. In the case of phyA SA, proportion of the pools was, however, substantially shifted towards phyA'' (up to 20/80%) and it did not depend on the presence of the endogenous phyB and on the duration of the light pretreatment. This strongly suggests that phyA SA comprises primarily or exclusively the phyA'' pool and supports the notion that the two phyA types differ by the state of serine phosphorylation at NTE. The differences in the phyA'/phyA'' composition in phyA SA as compared with phyA WT and endogenous *Arabidopsis* phyA may explain, at least

partially, their different participation in HIR vs. VLFR and LFR observed earlier (Kneissl et al., 2008). In this work, germination of the phyA SA expressing lines was much more effective than that of the phyA WT expressing lines what may also relate to the fact that phyA SA comprises primarily or exclusively the dephosphorylated phyA'' species.
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The influence of heavy metals (Ni, Cu) on the growth of *Cladina stellaris* (Opiz) Brodo in experimental conditions

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Lichens are an important component of Northern plant communities forming a significant proportion of the total biomass of ground vegetation involved in the cycle of nutrients. They affect seed germination of plants, and also support hydrothermal regime of soil. Among ground-layer lichens *Cladina stellaris* (Opiz) Brodo prevails in undisturbed boreal forest and is a sensitive indicator of aerial technogenic pollution of the environment. Due to the physiological characteristics lichens absorb moisture, nutrients and heavy metals by entire surface of the thallus, and their mineral composition adequately reflects the level of air pollution. However, the processes of absorption of heavy metals into the thalli of lichens from polluted soils remain poorly understood. In this regard, the laboratory experiment was conducted.
Thalli of *Cl. stellaris* were collected in August 2015 in the background area remote 180 km from St.-Petersburg. They were cleaned of impurities and separated into individual thalli. Each thallus was measured by the length of the dying (grey) and living (greenish color) parts. Then the thalli were immersed in vessels containing 25 ml of solution of sulphate Ni or Cu with the metal concentration from 125 to 500 mg/l, where they has aged for 14 days. The experiment was conducted in 3 replications, the control was distilled water. At the end of the experiment each thallus was measured by the length of the dying and the living, and wet and dry parts. In dry parts of the thalli after dry ashing and dissolving the ash in HCl the content of Ni, Cu was determined by atomic absorption spectrometry. Mathematical data processing was performed in the program Statistica 10.0.
Analysis of variance of data showed that all measured parameters (length dying, living, wet, dry parts of the thallus, the total length of thallus, its weight) of the experimental and control thalli were not significantly different. During the experiment the dying parts of the thallus of the lichen significantly increased from 3.0 to 3.6 cm ($t=4.87$, $p=0.000003$), accordingly, length of living part of the thallus significantly decreased from 6.0 to 5.5 cm ($t=4.37$, $p=0.00002$), as in the control variant, and at different concentrations of Ni or Cu ions in solution. The length of the wet part of thalli average was 5.5±0.1 cm and was significantly higher ($t=15.7$, $p=0.000001$) than the dying part. Accordingly, the length of the dry portion of the thalli was significantly less ($t=15.6$, $p=0.000001$) than the living part, and its average value was equal to 3.5±0.1 cm. Therefore, it can be stated that the flow of water or salt solution is controlled by capillary forces, in which it involves not only the dying part of podeli performing exclusively the function of the capillaries, but also living part of thalli of the lichen.
The relative height of rise for salt solution, i.e. the proportion of the wet part of total length of thalli, was significantly higher ($t=2.1$, $p=0.039$) in comparison with distilled water, and it is respectively 61.7% and 52.8%. However, correlation analysis of the data showed no correlation between Ni or Cu ions concentration in solution and the height of its ascent at the thallus.
Chemical analysis of dry parts of lichen revealed no significant increase in the concentration of Ni or Cu in the experimental thalli in relation to control values. In other words, heavy metal ions under the action of capillary forces act only in the wet part of podeli, but they do not move in the upper part of thallus.
It is known that at elevated levels of heavy metals in the environment Ni ions move faster in the above-ground part and accumulated more in the upper part of higher plants compared to Cu ions. However, our experiment showed no differences in these parameters between Ni and Cu ions.
Thus, it can be concluded that the ions of heavy metals (Ni, Cu) did not affect the speed of the withering away of the lower part of the lichen, perhaps due to their short-term impact; the flow of saline solution into the thallus is controlled by capillary forces and does not depends on the concentration of metal ions in solution; in the upper part of podeli heavy metal ions do not get from the salt solution, as evidenced by the absence of their elevated concentrations in dry parts of thalli.

Conservation of biodiversity of natural plants covers of Smaller Caucasus

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The aim of the study: Tovuz forest region is rich and diverse in natural plant cover. We have collected a rich herbarium material in a variety of expeditionary campaigns; we have studied bio-ecological features of flora, relief area, the area of various species. To the formation of the climate of the region is influenced by winds from the South Caucasus (Transcaucasia), in the forest vegetation are located in several levels. In slopes of the mountains, forests, plains, summer pastures areas of Shamlig, Asrik, Agbulag, Kiran, Cheshmaly, Khatindjan, Boyuk Gishlag of Tovuz region are distinguished with various vegetations. It has been examined by us biodiversity of flora of these areas, distribution the species in particular composition of trees and shrubs. There are identified bio-ecological features of the vegetation of the area. Soil composition is newly formed mountain-meadow, forest and sulfuric forest soil species. Development and distribution of vegetation zones depends on the soil.
Material and methods: Plant cover has been influenced by ecological condition and soil structure in formation. At the levels plant cover dense are reduced and there are observed plant species rejuvenation. In comparative study of plant's growth and development are observed difference according to fruits and formation of leaves. In the territory of Shamlig, Asrik, Boyuk Gishlag of Tovuz region are met with 4 species of *Euonymus L.* At the south slope are existed brown forest soil and there are existed some species of trees and shrubs: *Tilia L, Pyrus L, Ulmus L., Fraxinus L., Acer L., Mespilus L., Carpinus L., Quercus L., Clematis L., Hedera L., Vitus L. et.c.* There are mixed alpine and subalpine meadows. In some spaces are appeared impenetrable forests. Some species are from Azerbaijan flora – *Taxus baccata L., Pinus Kochiana, Klotzch in C.Coch, Cotoneaster saxatilis Pojark* et.c. They are existed in some limited areas and in our observation. Between drought resistant species are distributed mezofit species, basement of forest are consisted *Elaeagnus L., Ulmus L., Rhamnus, Cotoneaster L., Pyurus L., Rhus L., Cotinus Adans, Tamarix L., Morus L., Pyracantha Roem., Rosa L., Rubus L., Euonymus L. Mespilus L* et.c. From climbing plants are distributed *Clematis L., Hedera L., and Vitus L.* et.c.
Results: There are prepared concrete measures on protection of representatives of higher spores, gymnosperms, angiosperms, flowers plants. For conservation of Genofund is organized seed bank, therefore rare and endangered species are included to the Red book of Azerbaijan Republic. The result of these researches is the inventory of middle and high mountain zones of Tovuz region. There are determined by expeditions the inadvisable and intensive forest cutting. As reason there are reduced areal of valuable species of trees and shrubs. Some species are changed the view of areals by our observations: *Quercus macranthera, Ulmus scabra Acer trautvetteri, Betula pendula, B.litvinovii* et.c.

Prunus cerasus L. leaves morphologo-biochemical reactions on a biotic stress.

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Plants environmental insult protection is provided with structural adjustment, specific features of an anatomic structure, physiological reactions and protective substances production. To identify the cherry breeds which are resistant to biotic environmental factors diagnostics and a possibility to forecast the genetic variability and the inheritance of physiologo-biochemical and morphologo-anatomic signs at early stages of selection process have special relevance. The leaf which is defining, directing and a plant activity processes regulating is a convenient object to receive the information indicators of a plant condition. The leaf sculpture surface plays an important role in fruit plants adaptation to biotic environmental factors (Vavilov, 1953; Shmalgauzen, 1968; Deveroll 1980; Pautov, Yakovlev, Kolodyazhnyj, 2002). The researches of Deveroll G. (1984), Dgigadlo M.I.and Dgigadlo E.N. (2010), Pyankov V.I. (2008), Zaprometov M.N. (1974), Kretovich V.L. (1980), Motyleva S.M. et all (2014) identify that the anatomic leaf structure (lamina thickness, epidermic cells pattern, cuticle nature); quantity index (stomatal number and size); biochemical features (antioxidant complex); electrobioluminiscence (Kirlian effect) and chemical elements concentration in the leaves are the marker characteristics and can be used to identify the resistance. The purpose of our work is to design the resistance early diagnostics technique using the complex of the leaves morphological, anatomical and biochemical characteristics of cherry genotypes with different resistance to Coccomyces blight. The

leaves of *Prunus Cerasus* L. 14 genotypes (non-resistant ones, medium resistant ones and Coccomyces blight resistance donors) were analyzed. The specific features of cherry leaves cuticle sculpture and anatomic features were examined via scanning electronic microscopy method. The chemical composition of inclusions (dendrites) was determined via energy dispersive spectrometry method. The chromotrographic profiles of the leaves ethanol extracts were determined via liquid chromatography method. Coccomyces blight resistant forms have less stomatos. Stomatos quantity reduction mitigates the risk of pathogene penetration inward the leaf. The upper and under epidermis thickness of the cherry resistant genotypes leaves 1,5 - 2,5 times higher than non-resistant ones. Solid inclusions (dendrites) are found under resistant genotypes cuticle. The thick cuticle and the dendrites layer are the barrier for infection penetration. It is determined that the dendrites contain potassium, calcium, manganese and ferrum. Qualitative and quantitative differences of phenol compound content in cherry leaves were determined. The important specific feature of resistant forms is chlorogenic acid, its quantity exceeds the acid content in Coccomyces blight non-resistant genotypes leaves several times. According to the statistics given by Kuznetsova A.P. and Shestakova V.V. (2010) it is the characteristic of the plant resistance to biotic environmental factors. The results confirm informative value of the suggested methods to determine the genotypes which are Coccomyces blight resistant. So, it is determined that *Prunus cerasus* L. resistance is demonstrated on different levels of the leaf organization and is connected not only with morphologo-anatomic characteristics (cuticle thickening, stomatal number decrease, dendrites formation), but with increased synthesis of chlorogenic acid which plays the role of signal molecule in other resistant reactions coordination. Using the suggested physicochemical methods it is possible to select the adaptive cherry plant forms on the early stages of selective process for the further selection. The statistically significant differences of morphologo-biochemical cherry leaves characteristics are determined.

Cd induced growth and ion responses in leaves of amaranth plants

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The study of plants responses to Cd impact indicates its multiple damaging effects on plant organisms. However, plants are able to show tolerance to heavy metals, various aspects of which in relation to Cd has been discussed (Watanabe et al., 2009; Fan and Zhou, 2009; Bosiacki et al., 2013; Li et al., 2012; Ko et al., 2014, Chetan, Ami, 2015). One of the terms that determine the stability of plants functioning in control and under stress is ensuring homeostasis of internal parameters including ion homeostasis. Disturbances in the processes of mineral ions absorption and distribution in plants when exposed to Cd may be responsible for plant growth depression (Huang et al., 2007; Metwally et al., 2005; Nazar, 2012), while maintaining ion homeostasis in roots and aboveground parts is essential for ensuring plant tolerance to Cd exposure (Liu et al. , 2012; Vahedi et al., 2013). Studies in this area show conflicting results. The aim of our research was to assess the impact of Cd on pools of mineral cations and organic acids in leaves of plants depending on the age of the leaf. The object of the research were 42-days *Amaranthus cruentus* plants, grown in a hydroponic culture and subjected to 1, 10 and 30 μM Cd exposure for 7 days.

The results showed differences in responses to Cd stress in leaves of different age. In mature leaves a marked inhibition of growth processes was observed, whereas in juvenile leaves, on the contrary, there was an increase of biomass growth by 38% and 21% relative to the control at 1 and 10 μm of Cd. The ambiguity of Cd effect was evident in relation to K^+ concentration in leaves that was increased in the juvenile leaves by 21% and 25% with no effect in mature leaves. The contents of Ca^{2+} and Mg^{2+} also increased, largely in juvenile leaves, reaching 40% to control at 10 μm Cd. These data suggest that Cd stress induced the transport of cations to the shoot, possibly through the induction of transcription of the channels responsible for K^+ and divalent cations loading to the xylem, which may be associated with the work of adaptive mechanisms to maintain ion homeostasis in leaves of different ages. The differences were found as well in metabolic responses of mature and juvenile leaves on Cd exposure. While the increase in the pools of several organic acids, including malic, oxalic, fumaric acids was observed in all leaves, however, in juvenile leaves the response was much more intense than in mature, where the contents of succinate, in contrast, was declined.

Taken together the results allow to conclude that Cd in concentrations of 1 and 10 μM provoked in leaves of amaranth responses aimed at accelerating the flow of ontogenesis, one of the features are age-related changes in the ionic composition of leaves, including the increase in the content of organic acids and divalent cations which participate in their neutralization in vacuoles. As a signaling molecule, the involvement of ethylene as a stress hormone may be proposed that can determine the processes of

senescence in the form of aging process acceleration in mature donor sheets and maturation process acceleration in the acceptor juvenile leaves.

Regulation of enzyme activities in response to different stimuli from prey in the carnivorous genus Nepenthes

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Carnivorous plants typically attract, capture and digest animal prey by modified leaves called traps to obtain nutrients. The corpse is dissolved by digestive enzymes produced by the plant itself or by mutualistic organisms. Species of the genus *Nepenthes* belong to the group of carnivorous plants with leaves that are composed of an assimilation part and a passive pitcher trap. The traps are filled with a digestive fluid to digest prey and produce various hydrolytic enzymes: chitinases, esterases, phosphatases, ribonucleases, and proteases. The most important proteases responsible for protein digestion are nepenthesins I and II. Information about the production and induction of these proteases in response to prey capture is still limited. In recent years it has been found that jasmonates play role in botanical carnivory which has evolved from plant defence mechanism. Electrical and jasmonate signalling is beneficial for regulation of enzyme expression in carnivorous plants with active trapping mechanism (*Drosera*, *Dionaea*). In passive pitcher plants electrical signals in the form of action potentials have never been reported and it is unknown what molecule or phytohorme is involved in digestive process. In our study we focused on detection and regulation of enzymatic activity and pH in response to different stimuli from prey (prey, chitin, protein, NH_4^+ etc.) and the role of jasmonates in this process. We measured phosphatase, phosphodiesterase, chitinase and proteolytic activities. Furthermore, we quantified the abundance of aspartic protease nepenthesin using the specific polyclonal antibody in digestive fluid. Our results show that the plant can recognize different stimuli from prey and trigger appropriate response in the form of digestive enzyme production.
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Picking on little brother: sibling seeds with a conflict

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Interactions among kin may occur via different forms of signaling or communication, allowing related individuals to either intensify or ameliorate their responses to each other. Kin versus non-kin interactions have been well-studied in animals; however, we know less about how kinship affects interactions among plants. I conducted experiments in which seedlings of Aegilops triuncialis from the same mother competed with each other, and compared these interactions to those between seedlings from different mother plants. Aegilops is an annual from the Mediterranean that is now one of the most invasive species in the world. Aegilops has dimorphic seeds that occur within the same dispersal unit and are very close to each other on the same rachis. These "large" and "small" sibling seeds are only separated by a thin layer of maternal tissue (lemma). There is evidence that these two seed morphs interact in ways that involve forms of signaling prior to germination (Dyer 2004). In my experiments, when fertilized, plants from large seeds of Aegilops suppressed seedlings from both sibling and non-sibling small seeds; but plants from sibling small seeds were suppressed nearly twice as much as plants from non-sibling small seeds. When fertilized, plants grown from small seeds had no effect on plants grown from sibling or non-sibling seeds. When not fertilized, plants were much smaller, but the sibling-related effects of seedlings from large seeds were similar to those in the fertilized treatment. My results indicate that relatedness has the potential to affect how plants interact. Furthermore, this kinship generally intensified competitive interactions, especially for the effects of the dominant competitor. These results support the hypothesis that kin-related interactions among the seeds of Aegilops prior to

germination, reported by Dyer (2004), may serve to reduce strong competition among closely related individuals after germination.
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Regulation of chlorophyll biosynthesis in Norway spruce: role of light, ontogeny, temperature and oxygen

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Unlike angiosperms, gymnosperms have two mechanisms for reduction of protochlorophyllide to chlorophyllide, one that requires light catalyzed by light-dependent protochlorophyllide oxidoreductase (LPOR), and a second mechanism catalyzed by light-independent protochlorophyllide oxidoreductase (DPOR), which can synthesize chlorophyll in the dark. In this study, we examined the regulation of chlorophyll synthesis in response to light and temperature in different developmental stages (cotyledons and needles) of Norway spruce (*Picea abies*). The accumulation of chlorophyll and chlorophyll-binding proteins was strongly supressed in the secondary needles and in the cotyledons cultivated at low temperature (7°C). Whereas transcription of genes for DPOR subunits (*chlL*, *chlN*, *chlB*) and protein accumulation was deregulated in secondary needles, low temperature had only negligible effect on their transcription and translation, indicating post-translational control of chlorophyll biosynthesis at low temperature. Taking into account higher solubility of gases at low temperature and DPOR sensitivity to oxygen, we mimicked the low temperature by higher oxygen level in atmosphere. This resulted in etiolated phenotype of dark-grown seedlings, indicating participation of oxygen in regulating DPOR activity in spruce cotyledons. Although, the light decreased mRNA and protein levels of DPOR subunits, the aminolevulinic acid synthesis and the abundance of glutamyl-tRNA reductase (GluTR) is relatively high in the dark, indicating that phytochrome-regulation of gene expression is partially relaxed in gymnosperm seedlings.

The effects of wounding-associated memories on the regeneration of stems in flax (*Linum usitatissimum*) seedlings

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Plants, though sessile by nature, are capable of acutely sensing their environment. Rudolf Dostál (1967) showed that when young flax plants are injured, their growth pattern changes when subjected to different stimuli. Later, Michel Thellier (2000) demonstrated that the reactions Dostál discovered involve the storage and retrieval of information. Thellier also showed that responses could be delayed in plants, suggesting the presence of memory. It is known that plants possess multiple kinds of memory such as epigenetic and immune, but Thellier's previously stated memory has not been identified as one of these. We set out to investigate whether this wounding growth memory might be affected by wounding hormones just as regular plant growth is affected by certain growth hormones. Therefore, we hypothesized that this kind of wounding memory is mediated by jasmonic acid. The value of jasmonic acid to plants in defense is well established, but its role with memory is not understood. In our first sets of experiments, we used flax seedlings (*Linum usitatissimum*) to repeat the previous experiments on this subject to verify reproducibility in our lab. For the third experiments, we discovered that an increased concentration of hormones prompts an intensified response. In the last part of our study, we demonstrate that flax wounding growth memory responds differently and with different intensities to other stimuli such as jasmonic acid and salicylic acid. Our goal is to discover the underlying mechanism for this memory. If we understand this mechanism though which flax remembers the wounding and

responds accordingly, we may be able to mimic the stress; thus opening opportunities for modification to allow plants to become more resistant when the real stress comes.

Plant cell walls and their potential protective role in abiotic stress

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Cadmium is a well-known environmental pollutant with distinctly toxic effects on plants. To minimize the detrimental effects of cadmium and its accumulation, plants have evolved detoxification mechanisms. Such mechanisms are mainly based on chelation and subcellular compartmentalization. The plant cell wall is an important cell feature that performs numerous essential functions. As the first barrier it prevents the input of toxic metals into the cell and plant organ, but this defense mechanism is still fully unknown. In this connection the effect of toxic cations on cell wall polysaccharides composition in roots of maize has been studied.
Zea mays L. as a member of the Poales, has peculiar primary cell walls of the type II distinguished by higher content of hemicelluloses (arabinoxylan, glucuronoarabinoxylan, arabinogalactan) and lower content of xyloglucans and pectin compared to dicots. A combination of extractions (hot water and alkali), chemical (content of uronic acids, phenolics, proteins), and analytical (HPLC) methods for the characterization of cell wall composition in plants treated with cadmium cations were used. Alkali extractions with significant changes in the composition were applied to a column of DEAE Sephadex for futher separation and purification. NMR analysis was used for better description of polysaccharide subextractions. The results indicate that the cell wall functions not only as a sink for toxic metal accumulation, but is under the metal stress also modified.
Better understanding of plant cell wall function in defense against toxic metals could point out plants utilizable in the green technique, phytoremediation, for cleaning up toxic metal-contaminated soils and water.
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Pioneers in Plant Signaling

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Greek and Roman philosophers and scientists like Aristoteles, Theophrastus and Plinius were already enthusiastic about the phenomena of plant movements and the signaling processes behind, in particular concerning Mimosa, the "shy plant". Theophrastus mentioned "…and it is thus possible for the wonderful Mimosa of Memphis (*Mimosa asperata* L. added by the author) to collapse on being touched – it wilts! But after a while it recovers in all its grandeur" (Theophrast hist. pl. IV, 2, 11). Aristoteles included the plants into his soul system as the basis for the soul of any other organism.
In the nineteenth and twentieth century, when sophisticated techniques like microscopy, time laps photography and electrophysiology were established, experimental approaches in the field of plant signaling came into flowering. Marcello Malpighi, Nehemia Grew, Matthias Schleiden and Theodor Schwann established the anatomical basis for a general view on plants and animals. It was Julius Sachs who investigated effects of light and gravity on plants and wrote in 1865 the first book on "Experimental Physiology of Plants". Eduard Strasburger published in 1875 his results and forecasting ideas related to pollen tube growth and fertilization. Wilhelm Pfeffer was the first who documented and explained rhythmic leave movements after time laps photography in 1898. In spite of the fact that electrical signals in plants were indicated already at the very beginning of the twentieth century, it was finally Jagadish Chandra Bose who in 1926 showed that electrical signals play an important role in "The Nervous Mechanism in Plants".
Finally, all these pioneers contributed remarkably to our recent topic plant signaling and behavior; some of their results and forecasting ideas have been recently confirmed by cellular, molecular and genetic approaches, others have been forgotten or even overseen.

SESSION 2

Ion Channels, Transporters and Electrophysiology

Modeling *Chara* action potential under salinity stress: similarities to animal Ca^{2+} signaling?

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Thiel and colleagues demonstrated conclusively that the all-or-none nature of *Chara* action potential (AP) is determined by formation of inositol trisphosphate (IP_3), which in turn releases Ca^{2+} from internal stores (1). The Ca^{2+}-activated Cl^- channels are the main agent of the depolarization phase of the AP. Once the Ca^{2+} is re-sequestered by the calcium pumps, the chloride conductance drops and together with depolarization-activated potassium conductance, the membrane potential difference (PD) returns to resting level. In *Chara* cells subjected to 50 mM NaCl medium, the AP duration increases from ~ 3 s to up to 30 s and the APs are often spontaneous. The lack of stimulating pulse and our faster data-logging speeds revealed a sharp positive spike at the beginning of each AP (similar spikes were also observed in APs generated with the cell in normal pond water). We hypothesize that *Chara* plasma membrane may contain transient receptor potential (TRP) channels, which can be activated by diacylglycerol (DAG) formed at the same time as IP_3 by hydrolysis of phospha-tidylinositol biphosphate. While TRP channels seem to be absent from genomes of land plants, they were found in some chlorophyte algae (2) and may be present in Characeae. The second messengers IP_3 and DAG mediate release of Ca^{2+} as well as rapid inflow of either Ca^{2+} or Na^+ from the outside through TRP channels in many animal systems. Cardiomyocytes, for instance, display AP of similar shape to Characeae with initial sharp spike due to opening of the TRP channels (3). Our simulation departs from the Thiel model (1), converting the Ca^{2+} concentration changes into change in membrane PD, to gain understanding into the increase of AP duration under salinity stress. The modeling will reveal if the Ca^{2+} pumps are affected by the rising Na^+ concentration in the cytoplasm. The recently sequenced *Chara* genome will be scanned for TRP channels and the initial spike will be modeled accordingly.

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Cation channels are sensors of ROS and oxidative stress in plants

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ROS are critically important for plants' life. They are generated by intracellular and extracellular mechanisms and accumulate in apoplastic space, the compartment with low antioxidant activities. Moderate generation of ROS is necessary for normal physiology, but their overproduction results in oxidative stress associated with damage and dysfunction of cell components (Demidchik, 2015, Environ Exp Bot). The question of sensing ROS is still open. It is proposed here that cation channels are one of prime targets of ROS in plants. They catalyse initial and very rapid sensing of ROS.

In the plasma membranes of lower and higher plants, ROS instantaneously activate two major classes of ion channels: Ca^{2+}-permeable nonselective cation channels (NSCCs) and K^+ outwardly-rectifying channels (KORs encoded by GORK). Activation of cation channels by ROS leads to dramatic influx of

Ca^{2+} for signalling and nutritional needs and K^+ loss (electrolyte leakage) inducing cell shrinkage, programmed cell death and autophagy. Ca^{2+} entry also rearranges actin cytoskeleton and modifies vesicular transport. ROS-activated ion channels reveal complex nature of activation, depending on the developmental stage and oxidative capacity of tested ROS. The transition metal binding centres have recently been identified in some members of cyclic nucleotide-gated channels, a subclass of NSCCs (Demidchik *et al*. 2014, JXB). These centres potentially produce hydroxyl radicals from H_2O_2 directly in the channel's macromolecule. Mutation in ROS-sensitive moieties in K^+ efflux GORK channel leads to decrease of ROS-sensing capacity, suggesting that distinct molecular groups are responsible for ROS sensing by ion channels. These moieties probably confer physiological properties related to ROS, such as programmed cell death and autophagy.
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Tip localized hyperpolarization activated Ca^{2+}-channels mediate pollen tube growth control via kinase-dependent anion channel regulation

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Fertilization as well as plant sexual reproduction in general and crop yield in particular rely on the successful transport of immobile sperm cells by the pollen tube (PT) to the female gametophyte. A tip focused cytosolic Ca^{2+}-gradient represents the major determinant for growth control and navigation of PTs. By simultaneously performing microelectrode based voltage-clamp measurements with live-cell Ca^{2+}- and anion imaging, we identified hyperpolarization-activated Ca^{2+}-channels (HACCs) localized at the PT apex. Activation of HACCs by hyperpolarization pulses was accompanied by (i) an increase in the apical cytosolic Ca^{2+}-concentration ($[Ca^{2+}]_{cyt}$), (ii) high anion channel activity and (iii) a decrease in the apical cytosolic anion concentration. HACC inhibition eliminated the $[Ca^{2+}]_{cyt}$ increase and abolished anion channel activity suggesting a Ca^{2+}-dependent protein kinase (CPK)-mediated mechanism of anion channel activation. Molecular and cell biology data together with electrophysiological analyses of anion channel- and CPK-loss of function mutants revealed a CPK2/20/6-dependent activation of SLAH3, ALMT12, ALMT13 and ALMT14 anion channels in growing Arabidopsis PTs. Interaction of anion channels and CPKs was demonstrated via anion current measurements and bimolecular fluorescence complementation in *Xenopus* oocytes. The observed growth retardation phenotypes in single and multiple loss-of function anion channel- and CPK mutants together corroborate the physiological significance of a kinase dependent Ca^{2+}-decoding pathway in controlling PT growth performance via anion channel activation.

The vacuolar channel TPK1 forms a complex with a regulatory kinase involved in ABA induced stomatal closure

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In response to drought, the hormone ABA causes lowered stomatal conductance. Stomata also close in response to elevated CO_2. In both cases, the vacuolar K channel TPK1 is involved, contributing to salt release from the guard cell vacuole. In spite of considerable detailed knowledge about trans plasma membrane fluxes, virtually nothing is known about the mechanism that couples stimuli such as ABA to

the guard cell tonoplast. We identified an LRR receptor kinase that phosphorylates AtTPK1 at the 14-3-3 binding domain in an ABA dependent manner. While kinase expression is predominantly at the plasma membrane, ABA appears to induce kinase endocytosis which promotes TPK1:kinase interaction at the tonoplast and hence phosphorylation. Loss of function in the kinase mimicked tpk1 phenotypes with respect to a delayed response after exposure to ABA and insensitivity to elevated CO_2. These results not only identify a TPK1 regulator but point to co-expression of the kinase in two different membranes and to a hitherto unknown trafficking pathway.

Neuroactive compounds and electrical signaling in "green axon" *Nitellopsis obtusa*

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The discovery of neuroactive compounds and electrical signaling in plants suggests that signaling by neurotransmitters in human brains has evolved from a signaling mechanism that existed prior to the divergence of plants and animals. Investigation of neurochemicals function in plants highlight understanding on not only how and what plants can perceive in their environment to adapt their behavior but also on brain evolution mechanisms. It is unclear whether these compounds play a metabolic or a signalling role. In the most of plant, investigations of cholinergic and glutamatergic system focus on detection and structural analysis of distinct components. Nevertheless, molecular studies alone do not provide information regarding signaling and physiological characteristics of ion transport systems in the intact cells.

Electrophysiological techniques are useful to determine the effect of neuroactive compounds on the activity of ion transport systems in plant membrane and reveal their involvement in signal transduction. To investigate the role of neurochemicals as a signalling molecules in plant kingdom we have used Characeaen as a model system. Cells of *Nitellopsis obtusa* stand as a well-characterized experimental system showing unique ability to generate a large action potential (AP). APs play an important role in signal transmission not only in animals but also in plants. In higher plants, it is difficult to isolate the electrical signal of a target cell from that of surrounding cells. The *Nitellopsis obtusa* provides an ideal model system for investigation of instantaneous extracellular effects of various neurochemicals on the generation of plant bioelectrical signals *in vivo*, thus contributing to understanding more complex laws of functionality, adaptation and information processing in higher plants and animals.

Our laboratory has long-lasting experience in recordings of electrical signals in plant cells by intracellular microelectrodes thus enabling investigation of neuroactive compounds influence on electrical signals generation at the cellular level. Electrophysiological response patterns and alterations of them were assessed in single *Nitellopsis obtusa* cells under the exposure of classical neurotransmitters. Conventional glass-microelectrode technique in current-clamp, voltage-clamp and patch-clamp modes were used for the registration and analysis of electrical parameters. Signal processing and high discretization frequency enable detail investigation of 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.

Increase in spontaneous activity after administration of several neurochemicals on AP, caused not only by depolarizing effect but also by increase in excitability of the cell was shown. In the ordinary conditions excitation threshold of the *Nitellopsis obtusa* cells was 90 ± 10 mV. Voltage clamp experiments revealed that neuroactive compounds reduced the excitation threshold in all investigated cells. Changes in current amplitude after application of neurochemicals indicate effect on ion channels involved in AP generation. Neurochemicals activated K^+ ion channels at the rest, Cl^- and Ca^{2+} ion channels after excitation and enhanced activation of H^+-ATPase.

The membrane potential dynamics after repetitively electrically triggered APs was explored for evaluation of neurochemical treatment impact on functionality of plasma membrane transport processes. Ion fluxes, amplitude, duration and dynamics of repetitively evoked electrical signals were influenced. It could be possible that two APs, separated by some critical time interval, have physiological meaning not only in *Dionaea* but in other plant species; keeping the cell in more depolarized state after the first AP after acetylcholine exposure would be physiologically beneficial.

We conclude that neurochemicals cause changes in membrane permeability and interfere with electrical cellular signalling pathway in plants.

Influence of gadolinium on the activity of ion channels in the liverwort *Marchantia polymorpha*

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A patch clamp study carried out on vacuoles of the cells from the liverwort *Marchantia polymorpha* allowed recording the activity of two types of ion channels - slow activating vacuolar channels (SV) and Cl^- permeable channels. SV channels were slowly activated by positive voltages and carried K^+ from the cytoplasm to the vacuole. The same direction of Cl^- flow was carried by Cl^- permeable channels recorded at negative voltages. These channels were fast activated and in symmetrical concentration of 100 mM KCl possessed higher conductance (54±1 pS at -60 mV, n=4) than SV channels (26±1 pS at +60 mV, n=6).
Since SV channels are permeable to monovalent and divalent cations, including Ca^{2+}, an effect of gadolinium - an inhibitor of calcium channels was examined. The influence of this inhibitor on single ion channel activity was studied in cytoplasm-out configuration. One of the effects observed after application of this inhibitor on the cytoplasm side of the membrane was complete blockage of SV channels activity. Gadolinium did not block Cl^- channels but evoked short-lived flickering of the channels during which irregular open states were observed. Conductance of the channels was dependent on the gadolinium concentration and was greater at the higher concentration of this inhibitor. The value of this parameter obtained from the amplitude histograms calculated from short burst of the channel activity recorded at -100 mV increased from 32 pS at 1 mM Gd^{3+} to 47 pS in the presence of 4 mM Gd^{3+}. The channels were completely blocked after replacement of Cl^- by gluconate - an impermeable anion, which confirmed their selectivity to Cl^-.
The results indicate that gadolinium is an effective inhibitor of SV channels and can be use to distinguish the activity of the channels from other types of ion channels in the plant vacuole. Blockage of SV channels by gadolinium suggest that the channels possess common features with calcium channels and therefore can participate in calcium signaling.
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Transmembrane potential changes in *Physcomitrella patens* evoked by cold and menthol – influence of plant hormones

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Most plants are sensitive to cold. The electrophsiological reaction of plant cells to cold is often observed in the form of transmembrane potential changes. Here we present microelectrode investigations of such changes evoked by a fast temperature drop or application of menthol and its dependence on plant hormones.
In leaf cells of *Physcomitrella patens*, cold and menthol evoked a different shape of membrane potential changes. Immediately after cooling of the plant, fast depolarization of the membrane was observed. The amplitude of these responses reached 133 ± 5 mV (n=7), and duration measured in half of the amplitude (T/2) amounted to 86 ± 14 s (n=7). In comparison with cold, menthol evoked two phases of depolarization which differed in terms of speed. The slow phase of depolarization was observed after application of menthol and the fast phase - after exceeding the threshold of excitation, whose value in standard conditions was higher (more positive) than ca. -105 mV. Moreover, after depolarization, a plateau of membrane potential was observed, which was characteristic for these responses. The amplitude of such membrane potential changes reached 92 ± 5 mV (n=7) and T/2 amounted 321 ± 16 s (n=6). In order to study the influence of plant hormones on membrane potential changes evoked by cold and menthol on plant hormones, we decided to use 50 μM auxin (IAA), 50 μM abscisic acid (ABA), 500 μM salicylic acid (SA), and 50 μM gibberellic acid (GA).
IAA was used to define participation of proton pumps in both types of responses. The threshold of excitation after IAA was rarely exceeded (in 3 from the 7 tested plants) and even though it occured, the amplitude of the responses (72 ± 2 mV, n=3) was lower than in standard conditions. Such a result

indicates that one of the conditions which is necessary to evoke responses after menthol is inhibition of the activity of proton pumps.
Three other inhibitors (ABA, GA and SA) were used because of their contribution in responses to cold. ABA, similar to earlier used IAA, caused partial inhibition of responses evoked by menthol, because full response was recorded only in 3 from the 13 tested plants and the amplitude of the responses was 86 ± 6 mV (n=3). The most significant effects of the last two hormones used were changes in the T/2 of the full responses. GA caused reduction of T/2 recorded after menthol and the value of this parameter amounted to 209 ± 43 s (n= 4); in turn, the T/2 of cold responses recorded in the presence of SA was higher than in standard conditions and reached 115 ± 13 (n=10).

Asparagine effect on single Characean cell action potential parameters

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Plant homologs to mammal nervous system ionotropic glutamate receptors (iGluRs) are glutamate-receptor-like receptors (GLRs). These receptors genes were first discovered in *A. thaliana* few decades ago. Since then, numerous researches considering GLR phylogeny and physiological functions were conducted focusing mainly on AtGLRs (*A. thaliana* GLRs) elucidating their function as sensors and mediators for multitude of exogenous and endogenous signals in plants. Based on AtGLR researches with wild-type, mutant *A. thaliana* plants and heterologous expression systems it was demonstrated that AtGLRs function as integral membrane proteins with a ligand-gated ion channel activity, mediating Ca^{2+} also other cations such as K^+ and Na^{2+} fluxes across membrane when activated by various ligands, mostly amino acids (Glu, Gly, Cys, Ala, Ser, Asn) or other agonists (e.g. glutathione). It is clear that glutamate itself increases cytoplasmic Ca^{2+} and elicits plasma membrane depolarization in dose dependent manner, but certain evidence that native GLR mediates these ion currents is still missing. It was also suggested that AtGLR form tetramers and can bind more than one agonist, resulting in receptors with broad agonist and activation profile.
GLR proteins where found in small radish (*Raphanus sativus*), rice (*Oryza sativa*), tomato (*Solanum lycopersicum*) and wild grass (*Echinochloa Crus-galli*) but only scarce number of electrophysiological investigations were conducted with other plant taxes, although distinct glutamate and other GLR agonists effect on electrical signals propagation (AP generation when exposed to e.g. glutamate) were demonstrated in liverworts and barley. GLR agonists induced electrical activity measured in lower plants represent complex action potentials (AP) in tissues and such AP parameters changes as amplitude where not effected by amino acid concentration in these experiments, thus single cell measurements are favorable. Characean cells is a robust model system for their explicit electrical signaling, aqueous habitation and size. We used single internodal Characean cells as a model system for investigation of exogenous asparagine effect on electrical signaling properties. Since Asn is one of agonists of AtGLRs our research focused on Asn (0,1-5 mM) effect on single, intact plant cell action potential parameters. Microelectrode technique - intracellular and extracellular glass electrode with extracellular silver-chloride electrodes in voltage and current clamp modes were applied. APW (artificial pond water) was used as control solution for obtaining control measurements of electrically generated APs and current-voltage relations, following half hour treatment of 0,1-5 mM L-Asparagine in APW solution. Investigated electrophysiological parameters were dynamics of rest potential, AP parameters: excitation threshold, peak value, amplitude, chlorine current dynamics and maximum values during AP after amino acid application.
Our investigation indicates that 0,1-5 mM Asn elicits membrane potential depolarization in dose dependent manner from up to 23% from control values. 5 mM of Asparagine induced spontaneous AP in third of investigated cells. Asn hyperpolarized AP threshold and increases AP amplitude (measured from threshold to peak value) from control values also in dose dependent manner. These findings indicate that asparagine interacts with plant cell membrane potential and cell excitability, increases AP amplitude and chlorine efflux. Our research elucidate amino acid impact and physiological outcomes in intact, functioning cell and indicates that amino acid contents are perceived and presented also in electrical signaling involving GLR.

Short- and long-distance electrical signaling in carnivorous plants.

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In response to biotic and abiotic stresses, the plants generate fast electrical signals that travel through the entire plant from the point of origin. At all sites that received the electrical signals, jasmonate initiates defence-responsive gene expression. The carnivorous plants with active trapping mechanisms use electrical and jasmonate signaling for formation of outer stomach and digestive enzyme secretion. But what happens if the carnivorous plants are exposed to abiotic stress in the form of wounding, which generates electrical signals? Can the carnivorous plant recognize electrical responses? Can we trigger false alarm by wounding and induce enzyme secretion? Is there a systemic response? In this lecture, I will try to show you, how are the carnivorous plants of the genus *Drosera* and *Dionaea* able to recognize different stimuli from prey and environment.

TPC1-based Ca^{2+} signaling: slow and steady wins the race

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TPC1 (tandem-pore cation channels) in plants were characterized electrophysiologically and known as SV (for Slow Vacuolar) channels long before their molecular identification. This channel is ubiquitely and in great numbers, several to dozens of thousands copies per vacuole, expressed in every tissue of terrestrial plants. The channel displays a large conductance and is permeable for physiologically relevant cations as K^+, Na^+, Ca^{2+}, and Mg^{2+} with a little preference. The boom around this channel was boosted by the fact that it turns to be activated by cytosolic Ca^{2+}, which shifts its voltage activation threshold to physiologically attainable tonoplast potentials. Thus, a feedforward activation mechanism was postulated, when the SV-mediated downhill vacuole-to-cytosol Ca^{2+} flux will cause a progressive SV activation (and a larger Ca^{2+} release). Yet, large central vacuole represents a non-exhaustive Ca^{2+} store, with a capacity well exceeding the Ca^{2+} buffering capacity of the surrounding cytosol. Consequently, the SV activity has to be tightly controlled well below the threshold, crossing of which makes the above suicide scenario possible. The clue is vacuolar Ca^{2+}, which stabilizes the SV channel in its closed state. This year, crystal structure of the *Arabidopsis* TPC1 was published and molecular mechanisms for the channel gating by cytosolic Ca^{2+}, voltage and vacuolar Ca^{2+} were revealed. Also, a detailed kinetic model exists, which describes the effects of vacuolar Ca^{2+} and Mg^{2+} on the voltage gating. In the absence of Mg^{2+} and neutral vacuolar pH the SV channel acts as a super-Ca^{2+} electrode, with a slope of 40 mV/decade. However, introduction of more physiological ionic conditions (acidic pH, presence of Na^+ and Mg^{2+} in the lumen) makes the SV channel almost insensitive to changes of vacuolar Ca^{2+} within its physiological range of concentrations. Thus, due to the "buffering" effect of vacuolar cations, the activity of SV channels is low and stable, and could be increased only by the increase of cytosolic Ca^{2+} to the levels of tens of ¦M. Predictions on the magnitude of SV-mediated Ca^{2+} flux came close to the results of direct measurements by non-invasive MIFE technique. Moreover, MIFE experiments demonstrate that TPC1-SV channels dominate Ca^{2+} release from the vacuole. Basing on the quantitative analysis of the SV gating by vacuolar and cytosolic cations and voltage, we came to the conclusion that the SV-TPC1 activation and respective vacuolar Ca^{2+}-release may only occur at "hot spots", where TPC1 cytosolic Ca^{2+} binding EF-hands are exposed to very high local Ca^{2+}, generated in a close (<100 nm) vicinity of an active Ca^{2+} permeable channel of other membrane (e.g. plasma membrane, ER) or neighboring SV channel. We obtained kinetic evidence that SV channels can form functional clusters, when opening of one channel affects the opening of another. This mechanism does not imply that TPC-1 channels may be involved into the local Ca^{2+} signaling only. Recent studies demonstrated that TPC1 channel is a key element in systemic Ca^{2+} signal, rapidly propagating along the root, subjected to salt stress, and leaves upon wounding or herbivorous attack. TPC1 activity is also essential in the stomata closure, dependent on the external Ca^{2+}. Thus, a working model may be proposed, when a self-propagated Ca^{2+} wave may be generated by the interplay between plasma membrane Ca^{2+}-permeable channels and vacuolar TPC1 channels. Importantly, multiple close contacts between the two membranes need to be present on the way, to ensure the generation of microdomains with high Ca^{2+}. Possible arrangements and interactions of Ca^{2+} signal with changes in ROS, membrane voltage, and pH will be discussed.

Stress-induced Rb^+ efflux from roots of *Arabidopsis thaliana* plants lacking functional K^+ outwardly-rectifying channel GORK

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Introduction. Potassium (K^+) is the most abundant macronutrient, which has electrical, osmotic and metabolic functions in plants. K^+ uptake in roots is crucial for plants; however, K^+ efflux also occurs in some physiological conditions. Loss of K^+ from roots is often induced by stresses, such as pathogens, salinity, freezing, oxidants and heavy metals. At the cellular level, K^+ efflux is caused by K^+ efflux channels which in roots of *Arabidopsis* are encoded by GORK or SKOR ion channels. These channels are activated by both depolarization and reactive oxygen species (ROS). Here we have designed an assay based on measurement of the radiorubidium efflux, which can be used in studies of the structure-function relationship of K^+ channels and determining of the channel's ROS-sensitive moieties.
The main aim of this study was to develop a system for measurement of $^{86}Rb^+$ efflux and investigate effects of NaCl, hydroxyl radicals and H_2O_2 on $^{86}Rb^+$ efflux in *Arabidopsis thaliana* L. roots.
Materials and methods. We used radiotracer $^{86}Rb^+$ ($t_{1/2}$=18.64 d) as chloride (Radioisotope Centre POLATOM, Poland). Labeling solution activity was 40 kBq ml^{-1}. Ten to fourteen-day-old seedlings of *Arabidopsis thaliana* L. 'WS-0' and KO lines of K^+ channel GORK were used. The activity accumulated by seedlings was measured using gamma counter with a large detector (diameter of 7 cm). Seedlings were placed in holders and transferred in the labeling solution. After 30 min, the $^{86}Rb^+$-loaded seedlings were removed from the solution and placed in isotope-free solution. Loss of $^{86}Rb^+$ was registered every 1-5 minutes. Stresses were applied to roots 5 min after beginning of $^{86}Rb^+$ efflux registration. The following stresses were examined: 1) 200 mM NaCl; 2) 1 mM Cu^{2+}, 1 mM L-ascorbic acid, 1 mM H_2O_2 (Cu/a); 3) 10 mM H_2O_2. Root weight was measured at the end of each experiment. Curves of time course of $^{86}Rb^+$ efflux were plotted and analysed.
Results. $^{86}Rb^+$ efflux revealed three distinctive phases: rapid phase (5 min), slow phase I (5-10 min) and slow phase II (10-25 min). First one corresponded to efflux of $^{86}Rb^+$ from cell wall (apoplastic space). Slow phases I and II were associated with the isotope efflux from intracellular stores (symplastic phases). $^{86}Rb^+$ efflux rates in control and under tested stresses were calculated for the slow phase I (mostly cytosolic). In WT plants, $^{86}Rb^+$ efflux rates increased by 5, 3 and 2,5 times in response to salinity, Cu/a stress and H_2O_2, respectively (as compared to control). In GORK KO lines, stress-induced $^{86}Rb^+$ rates were approximately half that of WT. Thiourea (specific scavenger of hydroxyl radicals) decreased NaCl- and hydroxyl-induced $^{86}Rb^+$ efflux rates by 15-20% while in WT this agents did not cause any changes in fluxes.
Conclusions. We have designed a system for an accurate measurement of the stress-induced $^{86}Rb^+$ efflux from roots of higher plants. We have found that NaCl, hydroxyl radicals and H_2O_2 significantly stimulate the $^{86}Rb^+$ efflux from root cells in *Arabidopsis thaliana.* GORK channels catalyse at least a half of this efflux.
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VP-induced changes in intra- and extracellular pH influence the light and dark stages of photosynthesis in two different pathways

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A system response to a local action of adverse factors includes generation and propagation of electrical signals in higher plants. Damaging stimuli cause variation potential (VP) that can induce a number of functional responses, including photosynthetic changes, which is possibly connected with proton signal in a cell. Analysis of the role of intra- and extracellular pH changes in VP-induced photosynthetic response in pea seedlings was the aim of this work.
It has been shown that leaf burning induced VP propagation which was accompanied with an increase of extracellular pH and decrease of intracellular pH. Furthermore, VP caused the photosynthetic changes including decline of CO_2 assimilation rate and raise of non-photochemical quenching of

fluorescence (*NPQ*). Comparison of average dynamics of the photosynthetic response and pH changes accompanying VP was performed to analyze the relation between these processes. It has been shown that dynamics of extracellular pH changes was quite similar to dynamics of the gas exchange response (correlation coefficient (*r)* was equal to –0.72). On the other hand, dynamics of intracellular pH changes differed significantly from that of the CO_2 assimilation changes (r = 0.31). On the contrary, high correlation coefficient between changes in intracellular pH and *NPQ* (r = –0.92) and considerable differences in dynamics of the extracellular pH and *NPQ* (r = 0.35) have been shown. These results can point to the presence of two components in the photosynthetic response caused by VP in pea seedlings. One of the components is determined mainly by the gas exchange changes and related to the extracellular pH shift; the second component is connected with the raise of the non-photochemical fluorescence quenching and the intracellular pH decline.

At the next stage of this work an additional theoretical analysis, based on a previously developed model of VP, was carried out. Analysis of the model showed that it describes the photosynthetic response near stimulation area, the disappearance of the response at long distance from stimulation area, as well as different shapes of dependences of CO_2 assimilation rate (ΔA_{CO2}) and *NPQ* (ΔNPQ) changes on VP magnitude. A monotone increase of ΔNPQ and two-stage changes (raise and subsequent decline) of ΔA_{CO2} with an increase in VP magnitude have been shown. This dependence corresponds with experimental data and confirms the presence of two components in photosynthetic response.

The obtained results reveal that VP-induced photosynthetic response in pea is connected with changes in the intra- and extracellular pH. Increase of the extracellular pH determines the component of response concerned with the inactivation of the dark stage of photosynthesis, while changes in the intracellular pH cause the development of the component independent from the dark stage activity.

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Brassinosteroids modify ion channel activities and induce elevation of cytosolic free Ca^{2+} in roots of higher plants

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Introduction. Brassinosteroids (BRs) are endogenous plant hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. Exogenous BRs can improve the quantity and quality of crops and ameliorates effects of stresses. Using native and synthetic analogues of BRs as a tool to improve plant yield seems to have a great potential for agriculture and biotechnology (Khripach V., 2000). BRs have been intensively investigated for their biosynthesis, distribution and physiological functions using classical physiological tests, analyses of mutants and transgenic plants (*Arabidopsis thaliana* plants constitutively expressing aequorin). Recent data indicate that BRs are also sensed by the plasma membrane system catalyzing increase in the cytosolic free Ca^{2+} (in leaves of *Arabidopsis thaliana*). Zhao *et al.* (2013) have shown that the BR-induced elevation in the cytosolic free Ca^{2+} is abolished in knockout line lacking functional brassinosteroid receptor and after treatment with Gd^{3+} (blocker of Ca^{2+}-permeable nonselective cation channels) (Zhao Y., 2013). Zhang *et al.* (2005) using suspension culture cells of *Arabidopsis* have found that anion channel currents were inhibited by both 28-homobrassionolide and 28-castasterone and outwardly-directed K^+ conductance was stimulated by 28-homobrassionolide but inhibited by 28-castasterone (Zhang Z., 2005).

The aim of this study was to examine possible effects of brassinosteroids on the plasma membrane cation conductances in plant cells and related Ca^{2+} driven signalling events. Standard patch-clamp and aequorin chemiluminometry techniques were used (Demidchik V., 2011).

Results. Here, we report the first electrophysiological characterisation of brassinosteroid-activated Ca^{2+}-permeable channels in higher plants. Wheat root protoplasts (tested by patch-clamping) and whole arabidopsis plants expressing Ca^{2+}-reporting protein, aequorin (analysed by chemiluminometry), were used in this study.

In the whole-cell patches (wheat root protoplasts), 1 μM 24-epibrassonolide, 28-homobrassionolide or 24-epicastasterone were applied exogenously. Only 24-epicastosterone modified transmembrane cation currents while 24-epibrassonolide and 28-homobrassionolide did not cause any reaction. Addition of 24-epicastosterone at cytosolic side through the patch-clamp pipette increased Ca^{2+} influx conductance, which demonstrated characteristics of depolarisation-activated Ca^{2+} channels. The pharmacological analyses have shown that brassinosteroid-activated Ca^{2+}-influx conductance was sensitive to inhibitors of Ca^{2+}-permeable cation channels. Blockers of K^+ channels did not inhibit this conductance.The plasma membrane conductance, which was activated by an endogenous 24-epicastosterone, showed bell-like shape with maximal activation at depolarisation voltages (bath: 20 mM Ca^{2+}). Labelling castosterone

(and its derivates) with BODIPY (using castosterone-BODIPY conjugates which were synthesised chemically) showed that castosterone (and its derivates) can be transferred to the cytosol both in intact roots and protoplasts. This confirms that the effect of 24-epicastosterone at the cytosolic face can potentially be observed in real plants.
We also tested the effect of different brassinosteroids on cytosolic free Ca^{2+}, using *Arabidopsis thaliana* plants constitutively expressing aequorin. Six brassionosteroids including brassinolide, castosterone, 24-epibrassonolide, 28-homobrassionolide, 24-epicastosterone and 28-homocastosterone were tested. All six brassionosteroids induced elevation of the cytosolic free Ca^{2+} in arabidopsis root cells. In the present study we demonstrated that 24-epicastosterone being more potent than 24-epibrassonolide and 28-homobrassionolide. 10 μM of exogenous BRs was the minimal concentration at which statistically significant changes of the cytosolic Ca^{2+} were observed.
Conclusions. The obtained results suggest that the plasma membrane of root cells contains the brassinosteroid-activated cation-permeable channels, which can be involved in cell ion homeostasis and signalling.
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Variation potential in higher plants: mechanisms of generation and propagation

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Plant response to external wounding is based on activation of a number of signaling systems. In spite of the fact, that one of the leading line in the reaction coordination belongs to electrophysiological system, little is known about its functioning and in particular the process of generation and propagation of variation potential (VP) – the transitional depolarization of cell membrane extending out the zone of local wounding. It has been already shown that long membrane depolarization at VP is based on a temporary inactivation of the proton pump of plasma membranes. However, there are many signs of participation of passive fluxes of Ca^{2+}, Cl^- and K^+ in VP formation. The worth-noting one is the registered drop in the membrane input resistance at variation potential generation, that is probably based on the ionic channels opening. It has also been shown, that calcium ions can play a key role in the process of VP depolarization generation and transformation of the electric reaction to functional changes of plant cells. Such increase in intracellular concentration of free calcium leads to an inactivation of the proton pump and activation of anionic channels. Moreover, possible long-termed open time of calcium channels may be the reason of the long-lived inactivation of H^+-ATPase and long membrane depolarization and be also connected with the type of the channels participating in the reaction propagation, which is also still widely discussed. The prevalent hypothesis of VP propagation is hydraulic one relating to existence of induced by localized damage hydraulic waves, which propagation stimulates generation of electric response in plant cells. According to another hypothesis electrical reaction is induced by migration of wound chemical substance from the zone of damaging. There is also a suggestion that the transmission of the wound substance through the xylem occurs as a result of wound induced mass flow. The propagation of the wound substance could probably be connected with increase of diffusion velocity (turbulent diffusion), which is induced by hydraulic wave. Studying of the mechanisms of variation potential propagation with the processes of the electrical reaction formation on separate cells may reveal the way of the VP transformation to plant physiological response, i.e changes of photosynthesis activity, respiration rate, gene expression etc.

Electrical networks in plants, fruits and seeds

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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. 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 discovery of memristors in the plant kingdom creates a new direction in the modeling and understanding of electrical phenomena in plant membrane structures. A memristor is a nonlinear element; its current-voltage characteristic is similar to that of a Lissajous pattern. Recently we found memristors as components of plasma membranes in many plants, fruits and seeds. The analysis of the presence of memristors in a bio-tissue is based on cyclic voltammetric characteristics where the memristor, a resistor with memory, should manifest itself. Tetraethylammonium chloride, an inhibitor of voltage gated K^+ channels, or NPPB, a blocker of voltage gated Cl^- and K^+ channels, transforms a memristor to a resistor in plant tissue. Uncouplers carbonylcyanide-3-chlorophenylhydrazone (CCCP) and carbonylcyanide-4-trifluoromethoxy-phenyl hydrazone (FCCP) decrease the amplitude of electrical responses at low and high frequencies of bipolar periodic electrostimulating waves. 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.

AtGLR3.7 as a regulator of growth and development in *Arabidopsis thaliana*

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The genome of *Arabidopsis thaliana* contains 20 genes encoding glutamate-receptor-like receptors (AtGLR) which can be grouped into three clades. Active receptor complexes are supposed to be comprised of four single subunits which form an ion channel similar to their animal homologues. Based on their pore-forming domains, these proteins are nowadays considered as essential elements in plant signal transduction by mediating Ca^{2+} signals across various cellular membranes (for recent review see Weiland et al. 2015). Regulations of plant growth and development as well as physiological processes seem to be achieved by amino acid-triggered variations in cytosolic calcium concentrations. Among others, GLRs participate in seed germination, the formation of the root architecture, photosynthetic processes, plant stress and defence reactions, as well as in long distance electrical signalling (Weiland et al. 2015).
AtGLR3.7 seems to be the only AtGLR in *Arabidopsis thaliana* which is expressed in every cell in the plant body (Roy et al. 2008). Members of clade III have already be reported to function in the root meristem. Our findings indicate also for AtGLR3.7 an activity in meristematic tissues not only in the root but also within the shoot of *Arabidopsis thaliana*. On the basis of growth experiments conducted on knockout and overexpression lines of this particular receptor, we hypothesize a positive regulation

of root growth and rosette expansion by AtGLR3.7. Furthermore, minor deviations in life cycle progressions are caused by reduced or elevated mRNA transcript levels of AtGLR3.7. This could imply a hormonal regulation of plant development through this glutamate receptor.

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An organellar potassium channel is involved in osmoregulation in the lower plant Chlamydomonas

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Fresh water protozoa and algae face hypotonic challenges in their living environment. Many of them employ a contractile vacuole (CV) system to uptake excessive water from the cytoplasm and expel it to the environment to achieve cellular homeostasis. Potassium, a major osmolyte in CV, is predicted to create higher osmolarity for water influx. Molecular mechanisms for K^+ permeation through plasma membrane have been well studied. How K^+ permeates organelles such as CV is not clear. Here, we show that a six-transmembrane K^+ channel KCN11 in *Chlamydomonas* is exclusively localized to CV. Ectopic expression of KCN11 in HEK293T cells results in voltage-gated K^+ channel activity. Disruption of the gene or mutation of key residues for K^+ permeability of the channel leads to dysfunction of cell osmoregulation in very hypotonic conditions. The contractile cycle is inhibited in the mutant cells with slower rate of CV swelling, leading to cell death. These data demonstrate a new role for six transmembrane potassium channels in the CV functioning and provide further insights into osmoregulation mediated by contractile vacuole.

SESSION 3

Organelle, Cell-to-Cell and Long-Distance Signalling

The mitochondrial pulsing as novel player in plant cell signaling system: modulation by cytoskeleton remodeling

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Mitochondria are well-known as multifaceted organelles, which play an essential role in cellular metabolism not only as regulated source of energy and 'building blocks' but due to their signaling function, especially under influence of environmental and endogenous stimuli. Owing to opened new possibilities in confocal fluorescent microscopy, non-invasive functional imaging of live-cell processes, including dynamics of signaling events, become possible to detect with high spatio-temporal resolutions in a real-time manner and at a single-organelle level. To date, there is striking stochastic phenomenon termed 'flickering' or 'pulsing' visualized as abrupt transient depolarization of mitochondrial inner membrane potential being, therefore, attributed to mitochondrial permeability transition. Such pulses are often coupled with another discrete dynamic events termed as ‘mitoflashes’ or reactive oxygen species (ROS) flashes. Despite different pharmacological manipulations, the triggering mechanisms of initiation of these phenomena remain obscure. Moreover, occurrence of discrete mitoflashes in individual plant mitochondria has not been yet clearly demonstrated at all. Objects-to-be-tested were etiolated early-growth seedlings of winter cereals (*Triticum aestivum* L., *Secale cereale* L.) undergone low positive temperatures as well as field plants during autumn acclimation and wintering. Using confocal laser-scanning microscopy in combination with the appropriate fluorescent dyes (TMRM and H_2DCF-DA), we first received a strong experimental evidence of reproducible pulses and mitoflashes for plant mitochondria displaying their inherent and universal feature in living cells. To elucidate a putative role of cytoskeleton in the regulation of the mitochondrial dynamic events, we investigated the influence of anti-actin (latrunculin B, 300 nM) and anti-microtubule (oryzalin, 10 μM) drugs on behavior of mitochondria. Both anti-cytoskeletal agents reversibly stopped moving of cytoskeleton-associated subpopulation of mitochondria due to arrest of cytoplasmic streaming, and this simultaneously evoked stimulation of the pulsing rate. For wheat coleoptile epidermal cells, we detected average rates of 3.3 and 7.4 pulses per 100 mitochondria per 1 min for untreated and latrunculin-treated samples, respectively. In rye cells, latrunculin-stimulating effect on the pulsing rate was more evident (up to 5-fold) in comparison with wheat ones that positively correlated with intensity of cytoplasmic streaming. We propose that cytoskeleton disassembling decreases intracellular ATP consumption leading to overreduction of the electron respiratory chain that, in turn, results in increase of mitochondrial dynamic incidents to avoid excessive ROS production. Interestingly, that added H_2O_2 (100 μM) induced the similar increase of the pulsing rates (up to 2) while the effect of oligomycin (6 μM), ATP-synthase blocker, was higher (3-fold). Cold treatment dropped mitochondrial pulsing activity; in this case latrunculin had marked pulsing-stimulating effect on rye cells in contrast with wheat ones. The latter is most probably caused by the relevant cytoskeleton reorganizations as well as different adaptive strategies of these species to temperature stresses. To sum up, the pulsing activity being inherent feature of mitochondrial dynamic behavior is regulated by cell metabolic and oxidative status directly and/or via cytoskeletal-mediated modulations and might serve as an integral optical 'readouts' reflecting functional stress-induced changes, and finally cell survivability.

Organophosphorus plant growth regulator prevents changes in the morphology of mitochondria in stressful conditions

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Recently, carried out the synthesis and screening of synthetic drugs, which in small and ultra-small concentrations activate the growth and improve the quality of the products obtained. To these drugs relates melamine salt of bis (oxymethyl)phosphinic acid ("Melaphen").
In nature the plant organism is exposed to not the one, but several environmental factors. We investigated the effect of the complex effect of water deficit and lowering the temperature to 14 ° C on the functional state of mitochondria pea seedlings, in conditions that occur in the early spring.
The morphology of mitochondria may reflect their functional state. One of the methods of recording changes of morphology of biological objects is the method of atomic force microscopy (AFM).
The work was performed on 6-days mitochondria pea seedlings (*Pisum Sativum* L), cultivar Alpha. The control group of seeds for 30 min. soaked in water and the experimental group – in 2×10^{-12} M solution of melaphen (MF). Seedlings were grown in the dark at 24 ° C. Half of the control group two days seedlings and the seedlings which had been treated by MF was transferred on dry filter paper in a chamber at 14 ° C. After 2 days the seedlings were returned into control conditions where they remained for the next two days at 24^0C. The second half of the seedlings of the control group remained on moist filter paper for 6 days. On the sixth day the mitochondria were isolated from epicotyls of seedlings of all studied groups.
AFM images mitochondria pea seedlings, which were subjected on the two-day water shortage at a temperature of 14 ° C differed significantly from control samples. If in the control group of seedling were observed as separate and divisible mitochondria but in water deficit conditions at 14 ° C occurred swelling of mitochondria and the number of dividing organelles significantly decreased.
Statistical analysis of AFM images mitochondria showed that the average volume of mitochondria in the control was 81.0 (μm) $^2\times$nm, and the shortage of water at 14 ° C resulted in increased mitochondrial seedlings to 115.1 (μm) $^2\times$nm, but a seed soaking 2×10^{-12}m melaphen solution prevents changes of mitochondrial morphology. Sizes of mitochondria (v) were approaching to control values (Vav = 86 (μm) $^2\times$nm). Comparing published data with data obtained in our experiment it can be assumed that the combined effect of temperature 14 ° and water deficit, probably led to an increase of ROS generation by pea seedlings mitochondria, followed by swelling of the mitochondria.
Indeed, in these conditions in the membranes of mitochondria observed activation of LPO. The intensity of the fluorescence of LPO products increased in 2.5 times 3. Soaking the seeds in a solution 2×10^{-12} M melaphen caused a decrease in the fluorescence intensity of the products of lipid peroxidation to almost control levels.
It can be assumed that the protective effect of the drug due to its anti-radical and anti-oxidant properties. According to our data, the rate constant of melaphen interaction with superoxide radicals was amounted to 1.67×10^4 and it is comparable with the rate constant of interaction with nitroblue tetrazolium, and the effective rate constant of the interactions melaphen with peroxyl radicals (k_7) in the oxidation of ethylbenzene (60^0) was $1.64\times10^6(Ms)^{-1}$. By preventing activation of lipid peroxidation, melaphen, apparently helps to preserve the functional state of mitochondria, which is reflected on the morphological characteristics of mitochondria.
There is also an opinion that melaphen which contains purine and a phosphine fragments in low concentrations acts as a regulatory ATP and is able to cause the activation of a number of signaling systems. At the molecular models we have shown that in the molecule of melaphen, there is a similarity in the arrangement of charges on the surface available for water molecules, in the field of purine groups with the adenine part of the ATP molecule.

On use of root segments to study water transfer

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Root segments excised from intact plants are widely used in studies of various aspects of metabolism including water exchange. Obviously segment excision causes stress and, in turn, it gives opportunity to study adaptive responses in plants. The results of such studies are widely presented in literature. With respect to water exchange studies on root segments, there are some restrictions, since the direct consequences of excision are disruption of xylem and phloem pathways of water solution transfer, and the drop of pressure in xylem, phloem and adjacent parenchyma cells. Therefore, the study of water exchange in segments is restricted, as a rule, by short-range transport. The results are obtained mainly using a NMR spin-echo method with pulsed magnetic field gradient. The study of segments of maize root absorbing zone using NMR PMFG method showed the decrease in intercellular efficient diffusion coefficient of water, D_{ef}, both in radial and axial directions of root segments, compared to those in intact roots. It was shown earlier that pressure affects the conductance of plasmodesmata (PD) and membrane permeability. Oparka showed, using the dye method, the direct effect of pressure gradient between adjacent cells on the total irreversible "emergency" blocking of PD. These results raise a question whether the decrease in intercellular diffusional water transfer in root segments is connected to PD blocking resulting from the excision of segments from maternal plants.
The scheme of the study is following. Using spin-echo NMR method the relaxation and diffusion decays of water magnetization are measured in intact roots and root segments of 7-day-old maize seedlings in norm and after cutting of the same sample into fragments 3 mm long while the placement of segments in the sample tube is not disturbed.
It is shown that cutting of segments into fragments does not result in any significant changes in spin-spin relaxation. This indicates the lack of visible water loss through cuts and absence of influence of cuts on the rate of transmembrane exchange through plasma membrane. The cutting results in the trend towards the acceleration of transcellular diffusional transfer instead of slowdown, which could be expected for PD blocking. Thus, the total blocking of PD during cutting is not among the factors causing the decrease in intercellular diffusional water transfer in root segments. So there is no reason to believe that water exchange in root segments reduces below the water exchange range occurring in intact roots. The obtained experimental results do not exclude the dynamic corridor of water transfer regulation by changes in PD conductance in response to excision. However, the main reasons for D_{ef} reduction in root segments are most likely the drop in water pressure and decrease in the gradient of water potential in the radial and axial directions of the root due to disrupting of water efflux along the xylem.
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Tobacco Nt-4/1 protein can regulate antiviral RNA silencing

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The At-4/1 protein of *Arabidopsis thaliana* was initially identified as an interactor of the Tomato spotted wilt virus movement protein in yeast two-hybrid system. Subsequent studies of the Nt-4/1 protein, a *Nicotiana tabacum* ortholog of At-4/1, revealed that this protein is expressed predominantly in cells of plant vascular system and can influence the systemic transport of viroid infection. The Nt-4/1 protein has been previously found to bind RNA, showing a strong preference for imperfect RNA duplexes. The Nt-4/1 protein, being merely alpha-helical, was predicted to contain five coiled-coil domains and exhibit a tertiary structure similarity to yeast protein She2p, which is a RNA-binding protein involved in directed mRNA transport in yeast cells. However, the role of 4/1 protein in plants still remains unclear. To analyze the effect of high-level Nt-4/1 expression on viral infection and a possible role of the Nt-4/1 protein in antiviral response, *Nicotiana benthamiana* plants were inoculated with a Potato virus X (PVX)-based vector carrying the Nt-4/1-gene. The control plants were inoculated with the PVX vector without an insert. The virus accumulation was analyzed by immunoblotting and reverse transcription-PCR. A week after inoculation, differences in the development of viral infection between experimental plants infected with PVX and PVX-Nt-4/1 were observed. In the PVX-Nt-4/1-infected plants, infection

symptoms emerged a few days later compared to PVX-infected plants. Additionally, while the upper leaves of PVX-infected plants exhibited typical PVX symptoms, the uppermost leaves of PVX-Nt-4/1-infected plants lacked visible symptoms of infection, showing therefore a 'recovery' phenotype known to result from a strong RNA silencing response blocking the systemic transport of viral infection. These findings suggest that the Nt-4/1 protein may functionally interact with components of the silencing system involved in antiviral response. This hypothesis is in agreement with the previously reported ability of Nt-4/1 and its deletion mutant Nd90 to bind RNA. Moreover, in gel-shift experiments we found that Nd90 can interact with miRNA precursors *in vitro*. Since the Nt-4/1 protein is predicted to be structurally similar to the yeast protein She2p, which exhibits the RNA-binding properties only in the form of a dimer, the ability of Nd90 for dimerization was tested. Using formaldehyde cross-linking of purified protein *in vitro* and subsequent mass spectrometry of products, Nd90 was shown to be able to form dimers, and the central protein region was found to be responsible for dimerization.
These findings support the hypothesis that the Nt-4/1 can influence the antiviral silencing suppression response by means of interaction with RNA components of RNA silencing machinery, presumably involved in systemic transport of RNA silencing signals.

Exosome-like structures in the intercellular space of wheat callus tissues

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Research of extracellular structures is of particular interest due to the fact that they carry out cell-to-cell interactions that underlie the majority of biological processes, and serve as transporters of signals and inductors mediating hormone and stress-induced reactions. In this connection, the structural investigations of intercellular spaces of plant tissues are very important. The aim of this work is to study the ultrastructure of intercellular space of wheat embryogenic calli for the subject of presence the extracellular compounds and structures. By light and electron microscopy in the intercellular space of embryogenic callus strands extracellular polysaccharides and glycoproteins have been found. At the same time, not only fibrils and substances, but also the individual vesicles and structures were identified in the extracellular space. In particular, the dark large lipid droplets which are arranged both between cells and the cell walls, in vacuoles and cytoplasm. An interesting and novel result is that thin annular vesicles and large bright spherical corpuscles filled with fibrillar material were found in the extracellular space. These structures are expected to participate in cell-cell interactions of embryogenic calli and in the regulation of morphogenetic processes *in vitro*. Some of multivesicular structures are morphologically similar with exosomal complexes of animal cells, described in the intercellular space of animal cells.
Among a variety of vesicles released by cells, special attention is recently given to exosomes. Exosomes are defined as nano-vesicles of 50-100 nm in diameter and contain bilipid membrane and bound proteins, and cytosolic components, such as messengers RNAs and microRNAs (miRNA). Exosomes mediate communication between cells ferrying biologically active substances to distant cells and may contribute to changes in the genes and protein expression. They are quite stable in biological fluids due to the presence of the rigid membrane, which helps prevent splitting by hydrolytic enzymes. Plant exosomes are poorly studied, which cause the growing interest to the vesicles-mediated communication in plant cells.

Regulation of intercellular trafficking: the chloroplast connection

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Intercellular trafficking via plasmodesmata is an important route for signal transmission during plant growth and development. While the movement of water and small molecules like ions and sucrose has long been known to occur, more recent evidence suggests that larger molecules including proteins and small RNAs and even mRNAs traffic between plant cells. Given their essential roles, and the challenges of monitoring plasmodesmata and their functions, few mutants with defects in plasmodesmata have been described. Intriguingly, several of the few mutants that have been reported contain defects in

chloroplast proteins. The loss of one such protein, INCREASED SIZE EXCLUSION LIMIT (ISE)2, results in increased intercellular trafficking in *Arabidopsis thaliana* embryos and in *Nicotiana benthamiana* leaves. In these tissues with increased intercellular trafficking there is also increased numbers of twinned and branched plasmodesmata, likely the result of increased formation of plasmodesmata. ISE2 is a predicted to be an RNA helicase, and we have found that ISE2 is required for numerous aspects of RNA processing in these organelles, including rRNA processing, group II intron splicing and C-to-U editing. We also identified chloroplast RIBOSOMAL PROTEIN L15 (RPL15) as an in interacting partner of ISE2. Similar to *ise2* mutants, null *rpl15* mutants are embryonically lethal. Loss of RPL15 from *N. benthamiana* leaves leads to increased intercellular trafficking and increased formation of plasmodesmata, as observed on loss of ISE2. These observations suggested that defects in chloroplasts have non-specific effects on plasmodesmata. We therefore silenced expression of other chloroplast proteins with functions similar ISE2 and monitored intercellular trafficking in silenced tissues. We found that while reduced expression of these genes led to altered intercellular trafficking, the changes were specific to each silenced gene. These results suggests that specific chloroplast defects can exert unique effects on plasmodesmata-mediated intercellular trafficking.

The expression of plastid-encoded genes is differentially regulated by heat shock

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As sessile organisms, land plants have evolved special mechanisms ensuring their survival under unfavorable environmental conditions. Despite the numerous efforts to understand the molecular basis of heat stress tolerance little is known about the role of the plastid genome in temperature responses. In order to gain a better understanding of the principal effects of elevated temperatures on chloroplast gene expression we studied the temporal dynamics of transcript accumulation in *Arabidopsis* under heat shock. The expression profiles of both photosynthetic and “housekeeping” genes varied. A marked reduction of the amounts of the majority of the genes that we tested was detected during the first hour of heat treatment. We observed a 2-fold decline of *atpB* gene transcripts (for *β*-subunit of ATP synthase complex), as well as of transcripts of plastid $tRNA^{glu}$ belonging to a group of "household“ genes and required both for translation and chlorophyll synthesis. The expression of some chloroplast genes after suppression during the first hour of heat shock returned to steady-state levels. This group included the photosynthetic gene *rbcL*, encoding the large subunit of Rubisco and the "household” genes *rps14* (for the protein 14 of the small subunit of the ribosomes) and *rrn16* (for the ribosomal 16S RNA) involved in maintaining the functional state of chloroplasts. Hyperthermia induced a 2-3-fold reduction in the level photosynthetic genes *psaA* and *psaB* encoding apoproteins A1 and A2 of photosystem I reaction center. By contrast, the expression of *psbA* and *psbD* genes essential for PS II function (for proteins D1 and D2), began to rise after a reduction during the first hour and reached maximum values between 3 and 6 h of heat exposure. A more prolonged heat treatment resulted in their subsequent decrease to a 0.5-fold of initial level. These two genes, as well as *rbcL,* are mainly transcribed by plastid-encoded RNA polymerase (PEP). However, the expression of *rpoB* gene encoding *β*-subunit of PEP, and the *rpl16* gene of translation machinery were almost completely suppressed by the end of 24 h of heat stress. It is of interest that in *Arabidopsis* of the two photosystems only PSI is regulated at the level of transcription, while the expression of PSII gene *psbA* was shown to be controlled mainly by posttranscriptional mechanisms. Transcription of *rpoB* and the *rpl16*, in its turn, is performed exclusively by nuclear encoded RNA polymerases, RPOTp and RPOTmp. In our experiments the accumulation of transcripts of *RPOTp* and *RPOTmp* genes increased 2-4-fold after 3 h of heat treatment. Subsequently the level of *RPOTp* gene transcripts turned to initial value and later even decreased while that of *RPOTmp* remained at an elevated level throughout the 24 h of temperature treatment. These results suggest that inhibition of the expression of NEP-dependent plastid genes was not associated with changes in the levels of transcripts of nuclear encoded RNA polymerases and was possibly determined by the impairment in the work of other components of transcription machinery.

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Late flowering in chlorina mutants: how does lack of chlorophyll b affect flowering time?

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The reproductive success of plants depends to a large extent on the proper timing of flowering. The onset of flowering is under control of multiple endogenous and ecological factors, andlight plays a special role. The ecological signals are perceived by leaves, while the plant response (i.e., transformation of the shoot vegetative meristem to a floral meristem) occurs in the shoot apex. Signal transduction from leaves to apices involves the symplasmic route, as the signal molecules inducing synthesis of the FT transcription factor, the "florigen", as well as FT itself, move *via* plasmodesmata and through the phloem. In this context, the delay of the onset of flowering in a range of *chlorina* mutants with disturbances in the biosynthesis of chlorophyll *b* is a well-known but not investigated phenomenon. While superstabilization of the photosynthetic antenna *via* excess accumulation of chlorophyll *b* was shown to cause the delay in the onset of senescence (Sakuraba et al., 2010; 2014), we proposed that destabilized pigment-protein complexes of the photosynthetic apparatus in *chlorina* mutants can be a source of signals interfering with the timely onset of flowering. In the present study, *chlorina* mutants lacking chlorophyll *b* due to a null mutation in the gene coding for chlorophyllide a oxygenase - barley *chlorina f*2-3613 (Mueller et al., 2012) and *Arabidopsis ch*1-3 (Espineda et al., 1999) - were studied using cytological, biochemical and molecular methods. Protein components of the photosynthetic apparatus, generation of ROS, the expression levels of the marker genes of flowering and senescence were analyzed in leaves of wild type and mutant plants. The stability of antennae was estimated based on the rate of their degradation in the dark. The numbers of plasmodesmata between the mesophyll cells of the mutants were compared with those in wild type plants. Successful floral transition was judged of on the basis of morphological transformation of the shoot apices. The data allow to formulate the hypothesis that the increase in ROS generation in the *chlorina* mutants, which occurs due to destabilized antennae, leads to disturbed formation of plasmodesmata, which in turn results in defective transduction of signals necessary for timely floral transformation of the apices in mutant plants.

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Regulation of the transcription of plastid genes in *Arabidopsis* plants by brassinosteroid

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Because of the sedentary lifestyle, plants have multiple regulation systems determining their responses to external stimuli and adaptation to adverse conditions. Being the most important environmental factor, light has not only photosynthetic, but also regulatory functions. To fulfill the light development program controlled by photoreceptors plants form signal cascades including phytohormones as important elements of this system. Some plant hormones, such as cytokinins and especially brassinosteroids (BRS), are able to "imitate" the action of light, initiating typical light reactions, such as the expression of light-controlled genes or morphological changes in seedlings. The combining of these BRS-controlled processes results in the formation of a "light" phenotype in the absence of light. Further progress of studies in this direction can be connected with the study of the expression of light-controlled photosynthetic genes in plants differing in their endogenous BRS level.

To study the BRS-controlled regulation of the transcription of plastid genes, we used *Arabidopsis thaliana* plants differing in their endogenous BRS level; the parental line belonged to a Columbia ecotype with the normal endogenous BRS level, whereas its mutant form *det2* was characterized by defective

BRS synthesis. To achieve a high levels of BRS, detached rosette leaves of plants were treated with exogenous 24-epibrassinolide (EBL, 1 μM). Using the run-on method, we investigated the transcription of certain chloroplast genes in the lysates of intact chloroplasts from leaves collected from 3.5-week-old plants.

We compared the transcription rates of 12 chloroplast genes belonging to functionally different groups of plastome genes. First of all, they are the genes encoding the products that play an important role in the process of photosynthesis: the photosystem I genes *psaA* and *psaB*, the photosystem II genes *psbA*, *psbD*, and the *psbK*, gene of the large subunit of Rubisco (*rbcL*), the ATP-synthase complex gene *atpB*, and the subunit F of NADPH-plastoquinone oxidoreductase *ndhF*. Among the household genes, we investigated transcription of the gene encoding β-subunit of RNA-polymerase of bacterial type (*rpoB*), the genes of 16S and 23S ribosomal RNA (*rrn16* and *rrn23*), and the genes of tRNA-Glu and tRNA-Tyr (*trnE-Y*).

Comparative analysis of the intensity of transcription of chloroplast genes showed that the decrease in the endogenous BRS level observed in the mutant *det2* line promoted the activation of the transcription of the chloroplast genes studied. The highest (8- to 12-fold) activation level was observed for the *ndhF, psbK*, and *atpB* genes. A significant (3- to 4-fold) activation level was observed for the *psaA, psaB, psbA, rbcL, rrn23, trnEY*, and *rpoB* genes. The transcription of *psbD* and *rrn16* genes was activated less. Also, the exogenous EBL activated the transcription of some chloroplast genes. We observed a minor activation of the transcription of the most of the studied genes; in the case of five genes (*psaB, psbK, ndhF, rrn23,* and *atpB*); the difference was significant.

The data on the activation of the transcription of genes, required to realize the photosynthetic function of light, at the low level of endogenous BRS or under the influence of exogenous EBL, evidence that BRS are involved into the realization of the light program of plant development, starting from the changes in the level of expression of plastid genes.

Evolutionary aspects of non-cell-autonomous regulation in ancient taxa of vascular plants

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Multicellular organisms rely on cell-to-cell communications to achieve coordinated growth and development. Vascular plants use unique structures called plasmodesmata for this purpose. Plasmodesmata mediate intercellular exchange of a large number of macromolecules such as miRNA, proteins and mRNA. This fundamental role of plasmodesmata is well established in angiosperms but has not yet been traced back to the evolutionary ancient plant taxa. Comparative studies on representatives of gymnosperms, ferns and lycophytes could clarify the evolutionary history of non-cell-autonomous regulation in plants. The *KNOX* genes code for non-cell-autonomous homeodomain transcription factors that function as regulators of apical meristems. This function of the *KNOX* genes is well documented in a large number of angiosperms, and also in some representatives of other taxa. Thus, information on expression and localization of the KNOX proteins can be used for characterization of cell-to-cell transport via plasmodesmata in a large number of plant taxa. Patterns of localization of *KNOX* transcripts, KNOX proteins and plasmodesmata within shoot apical meristems of the representatives of different taxa can be analyzed using *in situ* RNA-RNA hybridization, immunolocalization of proteins and electron microscopy techniques, respectively. We report on the establishment of these methods for shoot apical meristems of the gymnosperm *Picea abies*, the fern *Ceratopteris richardii*, and the lycophytes *Huperzia selago* and *Selaginella kraussiana*.

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Receptor-mediated regulation of intercellular communication during pathogen attack

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Multicellularity depends upon the co-ordination of responses between cells and tissues. In plants, defence responses require the regulation of intercellular communication and this is mediated by plasmodesmata, membrane-lined pores that connect neighbouring cells. Plasmodesmata close and restrict intercellular molecular flux in response to the perception of a number of different pathogen associated molecular patterns (PAMPs). For chitin, this response is mediated by the LysM receptor protein LYM2 which is located at plasmodesmata. Significantly, this response is independent of chitin perception and signalling via the well-described chitin receptor kinase CERK1, indicating that the plasmodesmal membrane contains specialised signalling domains. It is well established that LysM receptor proteins and receptor kinases form complexes for the perception of ligands. Intercellular flux assays indicate that two additional LysM receptor kinases are required for chitin-triggered plasmodesmal closure, supporting the hypothesis that a specialised complex mediates plasmodesmal signalling. Both calcium and reactive oxygen species signalling act downstream of CERK1 mediated chitin perception and can trigger plasmodesmal responses. We are investigating their role in PAMP-triggered PD closure. Gene expression analysis indicates that chitin-triggered plasmodesmal closure positively regulates salicylic acid (SA) defence signalling, raising the possibility that SA distribution is controlled by the dynamics of the symplast. Pathogen-triggered plasmodesmal closure is common to a range of classes of pathogens, illustrating that regulation of cell-to-cell connectivity is a key element of host defence. Significantly, preliminary data suggests that pathogens suppress plasmodesmal responses to maintain symplastic connectivity in the host, confirming that control of the host symplast is also a key strategy for pathogen invasion.

Counter-directed changes in water transmembrane and symplast transport in plant roots as the features of systemic response to abiotic stresses

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The effect of stress factors on plants induces a number of defensive responses, both local in cells immediately contacting with effectors and systemic in cells distal from the treatment site. To reveal the mechanisms of plant abiotic stress adaptation, it is important to study early systemic responses concerning plant intercellular water exchange, and mobilized by cell-to-cell communications. We studied the dynamics of early changes in membrane permeability and symplast conductivity for water in intact plants under osmotic and oxidative stress. In the course of experiments the maize root tips were treated with various osmotic compounds (PEG-6000, NaCl, -0.5 MPa), hydrogen peroxide (6 mM) and using NMR method there was registered the diffusional water transfer in a root zone distal from treatment site in the time range from 0 to 4-5 hours after treatment.

The common early response to osmotic treatment was shown to be the increase in root transmembrane water transfer induced by aquaporin activity increase. This effect was reversible and was removed by aquaporin blockers of various nature (propionic acid, mercury chloride). There was obtained evidence in favor of counter directed regulation of aquaporin mediated transmembrane water transfer in roots at different levels of oxidative stress, which was controlled by lipid peroxidation level. The application of paramagnetic complex (Gd-DTPA) eliminating the apoplast water signal, demonstrated the inhibition of the symplast water transfer under osmotic and oxidative stress. The symplast transfer changes for intact and excised roots differed.

Thus, various stress factors induce in plants early systemic non-specific responses characterized by the increase in intercellular water transfer in roots due to the increased aquaporin activity, and by the decrease in symplast water transfer intensity. The difference in root water permeability dynamics in various cultures under osmotic stress depend on both original water permeability and the ability to switch the pathways of the root radial water flow (cell-to-cell, apoplast), and also can be a criterion for the choice of the strategy of response to water deficit and plant drought tolerance.

Cyclosis-mediated long distance communications of chloroplasts

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This work is devoted to the regulation mechanisms of light-dependent membrane processes of plant cell, such as photosynthesis, uneven distribution of plasmalemmal transport activity, and distant interchloroplast communications. As a model object, we used Characean algae that are able to generate under light the contrast patterns of external pH, calcification, CO_2 and O_2 content, as well as the patterns of photosynthetic electron-transport rates, non-photochemical quenching, and cytoplasmic pH. Internodal *Chara* cells could be as long as 10 cm and need effective mechanisms for signal transmission for long distances. Communications between chloroplasts and other organelles based on the exchange of metabolites, including redox active substances, are recognized as a part of intracellular regulation, chlororespiration, and defense against oxidative stress. Similar communications may operate between spatially distant chloroplasts in large cells where photosynthetic and respiratory activities are distributed unevenly under fluctuating patterned illumination. A comparatively new approach to investigate long-distance interactions between chloroplasts combines the localized illumination of a remote cell region with pH-microelectrode measurements and monitoring of chloroplast activity with PAM-microfluorimetry. We revealed large changes in actual chlorophyll fluorescence F′ (indicator of the acceptor Q_A redox state) and external pH at a distance of 1.5-5 mm downstream from the spot of bright localized illumination. The F′ profiles depend on the streaming velocity and disappear in the absence of streaming. Cytoplasmic streaming can be stopped temporarily by triggering the action potential with electrical stimulation of a cell. Depending on the exact timing of localized illumination and streaming cessation, kinetics of the F′ fluorescence response was characterized by positive or negative skewness. Our results indicate the importance of photosynthetic induction and the stromal redox state for long-distance communications of chloroplasts *in vivo*. Measurements of local light-induced profiles of chlorophyll fluorescence F′ at various distances from the LL source suggest that illuminated chloroplasts release into the streaming cytoplasm excess reducing equivalents that are entrained by the fluid flow and transiently reduce the intersystem electron carriers in chloroplasts of downstream shaded areas. We show that the metabolite transported with the cytoplasmic flow participates in early physicochemical events initiated by microperforation of the cell wall. The results present new evidence for long-distance transmission of signals with the cytoplasmic flow and for protective and regulatory role of this phenomenon.

A novel block of plant virus movement genes found in *Hibiscus Green Spot Virus*

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The success of plant viruses in establishing systemic infection largely depends on the virus ability for cell-to-cell transport. For their spread in plants, viruses exploit host transport systems involving plasmodesmata (PDs), channels that enable intercellular trafficking of small metabolites and macromolecules. Viral transport through PDs requires specialized virus-encoded movement proteins (MPs), which provide delivery of the viral genome to PDs, its translocation to neighboring cells and further long-distance systemic transport via the phloem. In different viruses, the MPs exhibit a remarkable diversity in their gene organization, as well as in their structure and biochemical properties. At the moment several types of viral transport systems are identified with the movement functions being performed by a single protein or distributed among several MPs.
In the present work we describe the transport gene module found in the genome of *Hibiscus green spot virus* (HGSV, the genus *Higrevirus*). HGSV is a recently discovered and so far poorly characterized plant virus with a positive-stranded RNA genome consisting of three RNAs designated as RNA1, RNA2 and RNA3. Three proteins potentially encoded by the HGSV RNA2 were reported to be distantly related to MPs encoded by a triple gene block (TGB), the conserved gene module responsible for transport of many viruses of the *Alphaflexiviridae*, *Betaflexiviridae* and *Virgaviridae* families and the genus *Benyvirus*.

To gain insight into the possible role of HGSV MPs in viral cell-to-cell movement, their coding sequences were cloned into a binary vector under the control of the 35S promoter of Cauliflower mosaic virus (CaMV) and examined in a transient cell-to-cell movement complementation assay employing a transport-deficient *Potato virus X* (PVX). Unlike the TGB, in which three encoded MPs are essential for viral transport, only two HGSV RNA2-encoded proteins were necessary and sufficient to mediate the PVX cell-to-cell movement.

To analyze the subcellular localization of HGSV MPs, their genes were translationally fused to genes of fluorescent marker proteins GFP and mRFP. Using the *Nicotiana benthamiana* transient expression system and confocal laser scanning microscopy, we analyzed subcellular localization of MPs expressed individually or in different combination with each other. Additionally, the GFP/mRFP fusions of HGSV MPs were co-expressed with fluorescent markers of specific cell sub-structures. Further, we examined the possibility of HGSV MPs co-transport to cell peripheral compartment, their translocation into the PD internal cavity and to the adjacent cells.

Taking together, our results indicate that the transport system of HGSV represents a novel movement gene module, extending therefore our understanding of the diversity of virus transport systems. Our experimental data are consistent with previous sequence analyses of virus-like RNAs found in the transcriptomes of *Lathyrus sativus* and *Litchi chinesis* that encode two proteins highly similar to the HGSV MPs proteins. The on-going analysis of the HGSV MPs can contribute to further disclosing the general principles of functioning of plant virus movement systems and their evolution.

Out of control: Stromal Ca^{2+}- binding proteins reveal the central role of Ca^{2+} in chloroplasts

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Calcium is an important secondary messenger in plant signalling. Chloroplasts are able to store large amounts of calcium, but not much is known about the role of calcium in these organelles. Chloroplast total and free calcium concentrations can vary considerably, calling for involvement of calcium binding proteins in regulating such a dynamics. As high calcium concentrations inhibit CO_2 fixation, it seems plausible that (stromal) calcium binding proteins play a key role in regulating calcium homeostasis in the chloroplast. We have previously identified two non-characterized chloroplast proteins we called LENA and LENB. Here, we show these proteins are not only localized in the chloroplast stroma and bind Ca^{2+}, we demonstrate further that they have a strong effect on thylakoid protein phosphorylation and double knockout or overexpressor plants exhibit slow growth and chlorosis under normal growth conditions. Moreover, *in vivo* analysis revealed that photosystem II is constitutively damaged in double-knock-out lines, while both overexpressors and double knock-outs showed a reduced photosynthetic electron transfer rate and altered thylakoid protein phosphorylation patterns, suggesting that LENA and LENB proteins play an important role in regulation of photosynthesis and most likely also other chloroplast processes.

Osmophoresis: a possible mechanism for vesicle trafficking in tip-growing cells

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Many cells drive through themselves polarized fluxes of ions and metabolites. In these cells, intracellular vesicles are exposed to cytosolic solution of non-uniform osmolarity. In such a solution, one pole of a vesicle experiences a higher external osmotic pressure than the opposite pole. This osmotic imbalance leads to water influx into the vesicle through its hemisphere facing the lower solute concentration and water efflux from the vesicle through its opposite hemisphere. The vesicle thus behaves as a jet engine propelled by osmotic pumping toward the region of lower solute concentration. An analysis will be

reported which shows that in tip-growing cells, particularly pollen tubes, the concentration gradient of solutes is high enough to drive vesicles in the cytoplasm either in the anterograde or retrograde direction, depending on the vesicle position, its radius and the phase of growth oscillation. The importance of intra- and transcellular water flow for cytoskeletal dynamics and cell motility will be highlighted.

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Cytokinin signal transduction is obviously initiated in the endoplasmic reticulum

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Cytokinin receptors were shown recently by different methods to be located mainly in the endoplasmic reticulum (ER). However, it remained unclear whether the ER-located receptors are active, especially as the presence of a minor part of receptors in the plasma membrane (PM) is not excluded. As ER and PM differ in composition and properties, the activity of receptors might depend on their local surrounding. However, binding properties of receptors (pH-dependence) indicates their inner membrane localization. We have, therefore, checked the functionality of receptors located in different membranes. Firstly, we tested receptor topology by a protease protection assay. The cytoplasmic part of the receptor of cytokinin receptor AHK3 was shown to be located in the cytosol and the hormone binding domain in the ER lumen. This topology is consistent with signal transduction from ER membranes. To check the subcellular localization of receptor-phosphotransmitter interaction *in planta* we performed BiFC experiments. Receptor and phosphotransmitter genes were fused with split eYFP sequences, expressed in *Nicotiana benthamiana* leaves and the subcellular localization of protein interaction was detected by confocal microscopy. We found that receptors interact with phosphotransmitters at the ER network and around nuclei, an interaction pattern being similar to receptor localization. This confirmed the topology experiment because eYFP part was located at the C-terminus (cytoplasmic part) of receptor, and phosphotrasmitters locate in cytosol and nuclei, so the interaction between receptor and phosphotrasmitter indicates the correct orientation of receptor in the ER membrane. Finally we tested the functionality of receptors in different membranes by an *in vitro* kinase assay visualizing the phosphorylation of phosphotransmitter proteins. Receptor genes were expressed in *N. benthamiana* leaves and ER and PM fractions were obtained from leaf homogenate by ultracentrifugation in a step sucrose gradient. Phosphotransmitters were obtained by expression of corresponding genes in *E. coli* followed by affinity column purification. Kinase assays were performed in a mixture of membranes, phosphotransmitters, and γ-^{32}P-ATP as substrate in the presence of various *trans*-zeatin concentrations, followed by SDS-PAGE and blotting onto PVDF membranes. We found that the major part of cytokinin-dependent kinase activity belonged to ER fractions indicating that ER-located receptors are active. Collectively, the data led us to conclude that the ER is a major site of cytokinin signaling initiation.

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In chloroplasts cadmium passes through envelope and stroma to reach thylakoids. Is it the finish?

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Cadmium is heavy metal which is very toxic both for plants an animals. It is widely used in different technologies which causes extensive cadmium pollution. Photosynthesis is important target for Cd action. The photosystem II is highly sensitive to Cd *in vitro*. Plants restrict Cd movement from soil to leaves. In leaves they bind Cd by cell wall polysaccharides. Inside leaf cells they transport Cd into vacuoles, trichomes or excrete it on a leaf surface. However Cd *in vivo* can inhibit photosynthetic processes.

The Cd effect on chloroplast function was not linked previously with its accumulation in chloroplasts. Two important questions are: 1) How many Cd can penetrate into chloroplasts? 2) In which chloroplast fraction – stroma or thylakoids – it is accumulated mainly?

The levels of Cd accumulation in chloroplasts of terrestrial plants and of diverse algal groups are incomparable. Only two research groups dealt with highly purified intact chloroplasts of terrestrial plants. They used different species, different experimental design and obtained drastically different results: rape chloroplasts accumulated 0.02 % of the leaf cadmium and common reed chloroplasts accumulated 10–15 % of the leaf cadmium. We studied Cd accumulation *in vivo* in chloroplasts of another two plant species. We obtained the intermediate values as compared to our predecessors. Maize chloroplasts accumulated 0.2-0.3% of the leaf cadmium, and barley chloroplasts accumulated 1.1-1.3% of the leaf cadmium (1). Our results filled enormous gap between previously obtained values and made obvious that Cd accumulation in chloroplasts of terrestrial plants can vary in a broad range.

We traced influence of Cd-induced stress on gene expression in chloroplasts and on photosynthetic function of photosystem II. We found only one parameter that correlates with Cd level in chloroplasts – photoinhibition of PSII (1).

Probably, we firstly studied Cd distribution inside chloroplasts. We used barley plants and the gentle method of chloroplast envelope disintegration (hyperosmotic shock). It occurs that in chloroplasts a less portion remains in stroma (and envelope) but a major portion is bound to thylakoids or translocated into thylakoidal lumen. This is in good agreement with previously obtained results (1): the level of mRNAs in chloroplast stroma was not changed and the level of proteins in thylakoids decreased in a concentration dependent level. We hypothesized that thylakoids may be not final repository of the introduced Cd but exclusion reservoir for further transport and probably signaling.

To verify the specificity of such a distribution we studied distribution of another two heavy metals – Cu and Fe – between stroma and thylakoids. Also we studied the effect of Cd-, Cu- or Fe-induced stress on the accumulation of essential cations Ca, Mg, Mn, Zn in leaves, chloroplasts, and their subcompartments - stroma and thylakoids. The results obtained will be discussed in the report.

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Subcellular localizations of Arabidopsis myotubularins MTM1 and MTM2 suggest possible functions in vesicular trafficking between ER and *cis*-Golgi

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The two Arabidopsis genes *AtMTM*1 and *AtMTM2* encode highly similar phosphoinositide 3-phosphatases from the myotubularin family. Despite the high-level conservation of structure and biochemical activities, their physiological roles have significantly diverged. The nature of a membrane and the concentrations of their membrane-anchored substrates (PtdIns3*P* or PtdIns3,5P_2) and/or products (PtdIns5*P* and PtdIns) are considered critical for determining the functional specificity of myotubularins. We have performed comprehensive analyses of the subcellular localization of AtMTM1 and AtMTM2 using a variety of specific constructs transiently expressed in *Nicotiana benthamiana* leaf epidermal cells under the control of 35S promoter. AtMTM1 co-localized preferentially with *cis*-Golgi membranes, while AtMTM2 associated predominantly with ER membranes. In a stark contrast with animal/human MTMs, neither AtMTM1 nor AtMTM2 co-localizes with early or late endosomes or with TGN/EE compartments, making them unlikely participants in the endosomal trafficking system. Localization of the AtMTM2 is sensitive to cold, osmotic and ionic stress challenges. In contrast to animal myotubularins, Arabidopsis myotubularins do not associate with endosomes. Our results suggest that Arabidopsis myotubularins play a role in the vesicular trafficking between ER exit sites and *cis*-Golgi elements. The significance of these results is discussed also in the context of stress biology and plant autophagy.

The functional role of the CLCe protein of *Arabidopsis thaliana* (L.) Heynh. in light-dependent acidification of thylakoid lumen

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The CLC protein family comprises anion channels and anion/H^+ antiporters. The *CLC* genes and their products were identified in all kingdoms and involved in a variety of physiological functions. Seven genes of the family, *CLCa-e,* were found in *Arabidopsis*, with their products residing in various intracellular membranes. One of the least studied proteins of the CLC family is AtCLCe, a thylakoid protein. In a previous work, an attempt was undertaken to evaluate the functional role of AtCLCe in photosynthesis by investigation of induction of chlorophyll fluorescence in *clce* mutants and WT *A. thaliana* (Marmagne et al., 2007). The lower level of fluorescence in the mutants than in WT during the fast phase of fluorescence induction led authors to the suggestion that decreased anion permeability of thylakoid membrane results in changes of ion content of the thylakoid lumen.
In the present work, we evaluate the functional role of anion channel/transporter of the thylakoid membrane, AtCLCe, by studying the effect of the *clce-2* mutation on *A. thaliana* photosynthetic activity. For this purpose, we investigated photosynthesis by measuring parameters of chlorophyll fluorescence, P700 redox reactions and P515 electrochromic effect (the long wave shift of carotenoid absorption) in *clce-2* homozygotic mutant and WT (ecotype Col-0) plants. The measurements were performed by using a pulse fluorometer Dual-PAM-100 (Walz, Germany). The homozygotic mutant line was screened by PCR. The plants were grown in soil at 22°C and 12 h photoperiod. Before the measurements, the plants were kept for 3 h in the dark.
Magnitudes of maximal PSII quantum yield of chlorophyll fluorescence, Fv/Fm, coefficient of photochemical quenching, qP, effective PSII quantum yield, Y(II), and electron transport rate through PSII, ETR(II), differed little when the mutant was compared to WT. Nonphotochemical fluorescence quenching, NPQ, and quantum yield of regulated energy dissipation in PSII, Y(NPQ), reflecting energy dependent heat dissipation of excited chlorophyll increased in *clce-2* in comparison with WT. Coefficient of nonphotochemical quenching, qN, was higher and quantum yield of nonregulated energy dissipation, Y(NO), was lower in *clce-2* than in WT. With the use of the module (P515/535) of the dual-PAM-100 we estimated both components of H^+ electrochemical gradient, $\Delta\psi$ и ΔpH, generated across thylakoid membrane under light. Contribution of $\Delta\psi$ to H^+electrochemical gradient was higher and the contribution of ΔpH was lower in *clce-2* than in WT but the sum of $\Delta\psi$ и ΔpH was the same in *clce-2* and WT.
We suggested that the increase of $\Delta\psi/\Delta$pH ratio in the mutant is a result of decreased anion permeability of its thylakoid membrane. NPQ activation in *clce-2* likely provides the effective functioning of ETC, when anionic permeability of the thylakoids is reduced. The mechanism of the NPQ activation observed in *clce-2* mutant in parallel with decrease of ΔpH remains unknown.
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Studies on light induced interaction of violaxanthin de-epoxidase with thylakoid lipids

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Violaxanthin de-epoxidase (VDE) is a plant enzyme which catalyzes important photoprotective reaction i.e. conversion of violaxanthin (Vx) to epoxide-free xanthophyll – zeaxanthin. Four chemical factors are indispensable for VDE activity:violaxanthin, reduced ascorbate (AscH), lipids forming inverted hexagonal structures and pH of the medium below 5. Decrease in the pH value of thylakoid lumen under strong light is a signal for VDE activation. Activated VDE interacts with the main thylakoid lipid,

monogalactosyldiacylglycerol (MGDG) which forms inverted hexagonal structures, and in presence of violaxanthin and AscH the de-epoxidation reaction starts.
Up to now, studies on the interaction between above mentioned chemical factors determining the activity of VDE were troublesome. Here, we present results obtained with the use of a new technology - microscale thermophoresis, allowing the studies on molecular interactions. All experiments were performed on the Monolith NT.LabelFree instrument with an excitation wavelength of 280 nm and an emission of 360 nm. As first, we demonstrated that four tryptophan residues of VDE are sufficient to obtain excellent quality signal and, because of that, no additional fluorescent markers were necessary for the analysis of VDE interactions with other biomolecules. Subsequently, we applied this technique for the first time for analysis of binding strength of the VDE to MGDG and to other thylakoid lipids at two different pH values. We found that although VDE was clearly bound to MGDG at pH 5, no such a binding was observed at pH 7. Additionally, at pH 5 VDE has bound to MGDG more than four times stronger than to another thylakoid lipid, digalactosyldiacylglycerol (DGDG). It was also observed that both MGDG and DGDG generated changes in the size, charge and solvation entropy of the VDE molecules and caused that the movement of the enzyme molecules along the temperature gradient became faster. It might suggest that size of VDE molecules was smaller when concentration of these lipids was higher, and this effect was more pronounce with MGDG comparing to DGDG.. Opposite results were obtained if galactolipids were replaced with two negatively charged thylakoid lipids i.e. sulphochinovosyldiacylglycerol (SQDG) or phosphatidylglycerol (PG). Movement of VDE molecules in the temperature gradient was slower with increasing concentrations of these lipids. This may suggest an increase of hydration diameter of VDE molecules and correlates with inhibition of VDE activity, what was described for SGDG presence.
The microscale thermophoresis measurements revealed also a strong effect of MGDG concentration on interactions between VDE and violaxanthin (Vx). It was found that at pH 7 and with MGDG presence in the buffer, VDE bounded Vx almost 6 times less efficient than in absence of this lipid. In contrary, at pH 5 the higher MGDG concentration facilitates better binding of Vx to VDE. Three MGDG concentrations were tested when Vx was used as VDE ligand at pH 5. At the lowest MGDG concentration which was 1.6 μM, increase of the Vx concentration resulted with decrease of VDE mobility along the temperature gradient. This may indicate VDE aggregation under Vx concentration above 10 μM. At two higher MGDG concentrations the increase of the VDE mobility was observed in whole tested range of Vx concentration. On the basis of MGDG and Vx effect on VDE mobility three different ratios of Vx for each initial MGDG concentration were selected to test the Vx de-epoxidation kinetics in *in vitro* system with HPLC method. Good correlations between results of microscale thermophoresis and HPLC kinetics studies were obtained.

Analysis of molecular interactions involving the tobacco protein Nt-4/1

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The *Nicotiana tabacum* Nt-4/1 protein is a plant-specific protein of unknown function. Earlier subcellular localization studies of the Nt-4/1-GFP fusion protein revealed its localization to cytoplasmic bodies co-aligned with actin filaments and capable of actin-dependent intracellular movement. The Nt-4/1 bodies, being non-membrane structures, were found in association with the plasma membrane, the tubular endoplasmic reticulum and endosome-like structures. Additionally, the Nt-4/1 protein was shown to be capable of nuclear-cytoplasmic transport. Previous analysis of bacterially expressed Nt-4/1 protein *in vitro* revealed that the protein secondary structure is mostly alpha-helical and suggested that it could consist of three structural domains. The Nt-4/1 protein was predicted to contain five coiled-coil domains, which might be involved in protein-protein interactions.
To identify possible protein interaction partners of the Nt-4/1 protein, a *N. tabacum* cDNA library was screened in a yeast two-hybrid system using a fragment of Nt-4/1 as bait. In this assay, a protein NtBap31 showing sequence similarity to mammalian Bap31 protein was identified. The human HsBap31 is known to be localized to the endoplasmic reticulum membrane and to participate in the processes of secretory proteins export, protein quality control and apoptosis. However, biological properties and functions of plant Bap31 are yet to be determined. To confirm interactions between NtBap31 and Nt-4/1, Nt-4/1d90 and NtBap31-C proteins were used. Nt-4/1d90 is a previously described Nt-4/1 deletion mutant, which retains properties of the full-length protein, in particular its ability to bind RNA molecules, whereas NtBap31-C corresponds to the C-terminal hydrophilic domain of NtBap31. 6-His-tagged Nt-4/1d90 and NtBap31-C expressed in bacterial cell and affinity purified were found to form a

heterodimer in solution. Further, the interaction of Nt-4/1d90 and NtBap31-C was studied by use of surface plasmon resonance spectroscopy. The kinetic association and dissociation constants, as well as the equilibrium dissociation constant of the protein complex, were determined. To analyze the interaction of NtBap31 and Nt-4/1 *in vivo*, a fusion gene NtBap31-mRFP was constructed. Subcellular localization of NtBap31-mRFP expressed in leaf epidermis cells of *Nicotiana benthamiana* by agroinfiltration was analyzed employing laser scanning confocal microscopy. NtBap31-mRFP was found to be localized to the endoplasmic reticulum (ER) and fluorescent punctate structures associated with the cortical ER tubules. The fusion protein Nt-4/1d90-GFP, similarly to previously described GFP fusion of the full-length Nt-4/1, was localized to numerous cytoplasmic Nt-4/1 bodies. Co-expression of NtBap31-mRFP and Nt-4/1d90-GFP resulted in re-distribution of the later from the cytoplasmic bodies to cortical ER tubules containing NtBap31-mRFP. Thus, we conclude that NtBap31 protein is localized to the ER membranes and is able to interact with Nt-4/1 both *in vitro* and *in vivo*.

Differences in the induction curves of chlorophyll fluorescence in apple fruits and leaves

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Characteristics of the photosynthetic apparatus in plant leaves (molecular, physiological and biochemical) were studied in detail by many authors. However, the role of chloroplasts in functioning of green fruits and their significance in adaptive mechanisms of higher plants is insufficiently characterized. Comparison of functional and structural characteristics of leaves and fruits of the apple tree during development is an informative line of investigation in the plastids signaling during the plant adaptation under natural development and stress conditions.

In our experiments we used green apple (*Malus domestica*) fruits measuring 20–40 mm in diameter. Apple fruits were collected in the morning (at 18°C). Measurements were made with a PEA fluorimeter (Plant Efficiency Analyzer, Hansatech, England) using fruit surface sections 2–4 mm thick with an area of about 1.5 cm^2. Samples of leaves and fruits were adapted to darkness for 5 min and then illuminated with red light (maximum at 650 nm, intensity 1500 μmol/(m^2 s), light pulse duration 2 s).

The fluorescence induction curves of the apple leaf comprised three growth phases (O-J, J-I and I-P) in accordance with the results obtained on other plants. The main distinction in the induction curve of chlorophyll fluorescence of the fruit peels was the existence of the transitions O-K-J at room temperature. According to the literature, the K-peak was commonly observed on leaf samples after the heat treatment (40°C) and was associated with the decrease in quantum efficiency of PS2. This observation was ascribed to the damage of oxygen-evolving complex and the imbalance of electron influx and efflux at the PS2 level. In our experiments, the peak K was achieved without the heat treatment, in 800 μs after the onset of actinic light. Its appearance was not accompanied by the decrease in the quantum yield of electron transport in PS2. The values of F_v/F_m parameter in apple fruits were 0,708-0,886 and were similar to the photosynthetic activity of the leaves.

The differences in fluorescence induction curves for fruits and leaves might be related to the gas transfer paths inside the green skin of the fruits. In contrast to the leaf, the occurrence of strong cuticle on the upper surface of apple fruits may change the functioning of stomata.

According to the transmission electron microscopy, the chloroplasts in maturing apple fruits are localized in the subepidermal layer. In terms of the ultrastructural features, these chloroplasts are distinguished by the developed membrane system, apparent regions of chloroplast contacts with each other, numerous occurrences of fusion and fission. In contrast to cells in the fruit skin, the leaf cells of apple trees contain abundant peroxisomes, which are often integrated into the "triads": mitochondria – peroxisome – chloroplast.

Evaluation of plant resistance to environmental factors using methods of chlorophyll fluorescence induction and electron microscopy can be used to study the adaptive role of plastid signals during stress.

Binding of the second messenger Zeaxanthin upon high light stress Changes the functional properties of the LHCSR1 protein from *Physcomitrella patens*

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Plants harvest photons for photosynthesis using light-harvesting complexes (LHCs)-an array of chlorophyll proteins that can reversibly switch from harvesting to energy-dissipation mode to prevent over-excitation and damage of the photosynthetic apparatus (1). In unicellular algae and lower plants this process requires the LHCSR proteins which transduce the high light stress into activation of photoprotective mechanisms by two synergic pathways: (i) by sensing over-acidification of the thylakoid lumen through protonatable residues exposed to the thylakoid lumen and (b) by activating Violaxanthin de-epoxidase (VDE), also pH sensitive (2). VDE transforms violaxanthin into zeaxanthin, which, in turn binds to the LHCSR1 protein, thus activating quenching reactions (3). In order to study the effect os the second messenger binding to the effector protein LHCSR1, we have produced the LHCSR1 protein of *P. patens* (PpLHCSR1) by overexpression in tobacco (4) and purified it in either its violaxanthin- or the zeaxanthin-binding form with the aim of analyzing their spectroscopic properties at either neutral or acidic pH. By using steady state and time-resolved fast spectroscopic methods we show that acidification of the medium does decrease fluorescence lifetime of the PpLHCSR1 protein leading to the appearence of a <100 ps component, which is a suitable quencher for the light harvesting antenna of PSII (LHCII) thus efficiently competing with PSII charge separtion for excitation energy and preventing photooxidation. In *P. patens*, the accessory PSI antenna, LHCII, is also quenched by LHCSR1 (5). The binding of zeaxanthin caused the LHCSR1 protein to decrease it fluorescence lifetime further and more extensively, consistent with an increased sensitivity to low lumenal pH.

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A comparative analysis of Photosynthetic Light use efficiency Regulation Mechanisms from unicellular algae to higher plants through mosses

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All oxygenic photosynthetic organisms possess mechanisms for fine regulation. This regulation determines fraction of chlorophyll excited states which are channeled to reaction centers for fueling electron transport vs heat dissipation. All these mechanisms are dep4endent on carotenoid composition and yet the mechanism by which the heat dissipation is catalyzed is still under debate. Here, we report on two proteins involved in LUE (light use efficiency) regulation, namely LHCSR and PSBS, their chromophore requirement and functional properties. These proteins initiate the signal transduction pathway(s) which detects the absorption of excess energy by the photosynthetic apparatus that cannot be used by downstream metabolic reactions and therefore becomes available for ROS production and photoinhibition. In both PSBS and LHCSR the initial stimulus is lumen over-acidification and low pH is detected through protonation of glutamate and aspartate residues exposed to the lumen which are the target of the DCCD inhibitor (1, 2). Further steps down the signal transduction pathways diverge in

algae vs plants: the pH-dependent conformational change in LHCSR activates quenching reactions within the protein itself owing to their capacity of binding chlorophyll a and xanthophylls (5, 6), including zeaxanthin, a power activator of Non Photochemical Quenching (4). Quenched LHCSR further interacts with component of the antenna system of both PSI and PSII (5, 6) thus reducing their functional antenna size and extending heat dissipation to additional antenna domains. The case of PSBS is different in that the protein itself does not bind pigments and therefore cannot catalyze quenching reactions (7, 8). Its interaction with monomeric components of the antenna system, chiefly CP29, induces conformational changes which activates quenching reactions (9, 10). Additional interactions with LHCII are currently analyzed in order to verify their functional role. We suggest that the modes of regulation evolved with the light environment available in water vs terrestrial environments and with the spectroscopic properties of light harvesting systems.

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Role of ferredoxin redox state in chloroplasts adaptation to heat stress

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The effect of heat treatment (40°C, 3 h, illumination at 100 μmol m^{-2} s^{-1}) on the photosynthetic electron flows in barley seedlings of different age was investigated. Thermoinduced inhibition of the liner electron flow due to impairment of the water oxidizing complex (WOC) and the increase in the extent of Q_A^- reoxidation by $Tyr_{z\ ox}$ was shown by measurements of oxygen evolution and Q_A^- reoxidation kinetics in the absence and presence of exogenous electron acceptors. Using HPLC analysis, the decrease in size of the photoactive PQ-pool and a change in the proportions of oxidized and reduced PQ under heat treatment were shown. Analysis of light induced $P700^+$ kinetics shown limitation of electron flow to ferredoxin - NADP+ oxidoreductase under heating. On the other hand the decrease in cyclic ferredoxin-related electron flow was observed while alternative electron flow from stromal reluctant was accelerated by high temperature. Using artificial oxidizing agent to ferredoxin was shown that thermoinduced limitation of ferredoxin-related as linear as cyclic electron flows is caused by reduction of ferredoxins. The increase in ferredoxin oxidation level reduced thermoinduced limitation of electron flows on PSI acceptor side. Moreover changes in ferredoxin reduction level modified the redox state of plastoquinone pool. The artificial oxidation of ferredoxin activated redistribution of plastoquinone molecules between non-photoactive and photoactive pools. Following heating of leaves did not affect on redistribution of molecules between non-photoactive and photoactive pools. However plastoquinone reduction level decreased in pre-treatment by DPIP leaves under heat stress. Apparently, the oxidation of ferredoxin accelerated electron flow on PSI acceptor side, electron donation to $P700^+$ on donor side and intersystem electron flow. If plastoquinone is reoxidated rapidly the necessary in addition plastoqiunone molecules are not present and plastquinone redistribution are not observed under heating.

Defence sugarcane glycoproteins cause microtubular disorganization and defects in structure and migration of nuclei of *Sporisorium scitamineum* teliospores

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Resistant sugar cane plants naturally produce defence glycoproteins that accumulate in the parenchymatous cells of stalks and prevent the infection by the filamentous fungi *Sporisorium scitamineum*. These glycoproteins, which have been defined as macromolecules of medium and high molecular weight (MMMG, Mid-Molecular Mass Glycoproteins and HMMG, High-Molecular Mass Glycoproteins), induce chemotaxis, homotypic adhesion and inhibit teliospore germination.
The inhibitory effect on germination caused by sugar cane glycoproteins seems to be related to microtubular disorganization in fungal cells. During germination, nuclear migration occurs in parallel to a strong polarization of microtubules (MTs) at the basis of the hyphae. Tubulin polarization seems to be required for pathogen development since 8 μg mL^{-1} nocodazole reduces fungal germination in a 50 % after 15 hours of treatment. Interestingly, after contacting with sugar cane HMMG glycoproteins microtubule polarization in fungal cells does not take place, either. Microtubule immunolabeling images reveal a decrease of approximately 70% in the number of polarized cells in presence of glycoproteins produced by resistant varieties of sugar cane. Treated cells exhibit a homogeneous fluorescent immunolabeling whereas control teliospores show an evident crescent-shaped fluorescence distribution as a result of polarized microtubules. Similarities with nocodazole effects indicate that microtubules disorganization must be involved in a failed germination. Moreover, as a consequence of a non-correct arrangement of microtubules, teliospores exhibit nuclear and microtubular alterations after incubation with HMMG glycoproteins. Nuclei, which appear decondensed and fragmented, cannot correctly migrate through the growing hyphae and germination fails.
Arginase activity contained in defence glycoproteins is already described for preventing fungal germination. Now, its enzymatically active form is presented as a link between the defensive capacity of glycoproteins and the MT disorganization in fungal cells. Active arginase is constitutively produced by healthy resistant plants whereas it is not detected in the juice from susceptible varieties. That is why MT depolarization, nuclear disorganization as well as germination of teliospores are not significantly affected by glycoproteins from non-resistant plants. Susceptible to smut disease plants try to increase the arginase activity in juice after detecting the presence of the pathogen but it is "too late" for an effective response and plants are not able to defend themselves.

Mitochondria in plant cell cultures growth: energetics and signaling

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The localization of reactive oxygen species (ROS) in the cells of non-morphogenic callus of Tatar buckwheat, characterized by high growth rates was studied. By fluorescence and electron microscopy methods used the main sites of ROS localization and their change in passage were established. It was shown that during culture cycle ROS localized in the non-morphogenic callus cells around the nucleus, in the cytoplasm and in the cell wall. ROS in the cell walls are found throughout the culture cycle, while around the nuclei ROS are found mainly on the 3rd - 7th day of cultivation, when the culture is actively growing. It was found that the source of ROS-specific fluorescence around the nuclei is mitochondria. The study of cell ultrastructure revealed that the nuclei in non-morphogenic callus manifest a complex shape, with numerous deep grooves that are filled with mitochondria. Probably such arrangement of organelles provide a greater energy supply for nuclear biochemical processes such as DNA replication or/and transcriptional activity. It is also known that the movement of mitochondria to the nucleus may be associated with participation of ROS in regulatory processes, while ROS affect tubulin polymerization, mitotic spindle formation and nuclear envelope dynamics. ROS may be involved in the cell cycle regulation through redox-sensitive areas (e.g., cysteine residues) of some regulatory proteins such as MAP-kinases, although the exact mechanisms remain unclear. Probably in the non-

morphogenic callus of buckwheat ROS, formed as a result of the mitochondria action can also be involved in the regulation of cell division.
Thus, we assume that ROS in cells of non-morphogenic callus are formed as a result of active operation of the mitochondria required for energy-consuming processes in the polyploid nucleus and can be used for cell division processes.

Plant virus movement proteins encoded by triple gene block: functions, origin and evolutionary links

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Cell-to-cell movement of plant viruses occurs through plasmodesmata interconnecting cells in plant tissues and requires dedicated virus-encoded movement proteins (MPs). Viruses of many genera possess a transport system consisting of three MPs encoded by so-called 'triple gene block' (TGB). In addition to the TGB proteins, some of these viruses depend on viral capsid protein in both cell-to-cell and long-distance transport. The three TGB genes called TGB1, TGB2 and TGB3 partially overlap and encode proteins with characteristic features. The TGB1 protein comprises a helicase domain. In addition to the RNA helicase and NTPase activities, the TGB1 protein is able to bind single-stranded RNA and, in some viruses, to suppress RNA silencing. This protein is essential for formation of the transport form of viral genome, either ribonucleoproteins (RNPs) consisting of the TGB1 protein and viral genomic RNA in viruses with a "hordei-like" TGB, or filamentous virions modified by binding of TGB1 molecules to the virion end containing the 5'-terminus of viral genomic RNA, as found for viruses with a "potex-like" TGB. TGB2 and TGB3 are small proteins integrated into cell membranes due to hydrophobic sequence segments and required for the delivery of the TGB1 protein to plasmodesmata. The TGB3 proteins contains specific signals for plasmodesmata targeting and therefore can serve as a 'driving force' for the targeting of TGB2 and TGB1-containing movement-competent complexes (RNPs or modified virions) to plasmodesmata-associated domains of cortical endoplasmic reticulum. At a next step of viral cell-to-cell movement, enzymatic activities of TGB1 are required for the transport of viral genome into the plasmodesmata interior and further to neighboring cells. Recently, we hypothesized that the silencing suppression activity gained by a viral replicative helicase led to the emergence of the second "accessory" helicase possessing the activity of viral silencing suppressor and/or movement protein. This hypothesis accounted for the evolutionary origin of the TGB, and the sequence analysis of novel viral genomes supported the proposed scenario of TGB origin and evolution, which included the following steps. First, the accessory helicase gene could have been acquired by horizontal gene transfer presumably occurred independently in different virus groups. Second, the TGB2 gene evolved by horizontal gene transfer or autonomization of the C-terminal transmembrane domain found in at least one TGB1 helicase. Third, the TGB3 gene has most likely emerged in the genomic block consisting of the TGB1 and TGB2 genes. This scenario is consistent with our new experimental data on previously unstudied plant viruses.

Radial water transport and growth by elongation in the maize root under hyperbaric conditions

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Radial intercellular process of water transport in the plants root performs an important role in a single water system of plant water supply, and also in plant adaptation to various environmental changes. In addition to plant response to changing of environmental parameters such as, temperature, humidity, it is also interesting the plant reaction to absolute atmospheric pressure changes. What changes do occur in the intercellular water transfer and in the plant growth under external atmospheric pressure increasing? What is the limit of plants resistance to extreme pressures. Answers to these questions are the aim of the present work.
In plant tissues and in roots in particular, cell to cell water transfer occurs along two pathways: the transmembrane (mainly through aquaporins) and symplastic (via plasmodesmata). Depending on external conditions the contribution of these pathways to the total transfer of water can be different.

In this work, using the spin-echo NMR method with GdDTPA (0.025 M) contrasting agent reversible increasing of intercellular radial water transfer in the maize root and water permeability of symplastic and transmembrane root systems under pressure of 2 - 4 MPa was shown. This effect may be associated with the mechanical influence of pressure on water channels which can modify their conductivity. However, should be considered, that pressure may have an influence not only through mechanical action, but also through additional dilution of atmospheric gases in the plant cells. We have previously shown that under hyperbaric conditions the atmospheric oxygen fast enough to penetrate into the root cells. This process leads to hyperbaric oxygenation with possible changes in the intercellular water transport as a result of oxidative stress and lipid peroxidation of cell membranes. Exposure to hyperbaric conditions also affects the growth of plants. It is shown that decreasing of roots growth rate by pressure raising up to 2 MPa occurs after 5 hours with full growing stop approximately after 6-6.5 hours. However, after depressurization to atmospheric level root growth is resumed for one hour. Decreasing of root growth rate likely associated with growth inhibition by elongation, as since inhibition of cell division is observed at much higher pressures. Influence of 4 MPa pressure during 5 hours leads to a more appreciable reduction in the growth rate, but this "dose" of the impact is not lethal for the plant. A further increasing of exposure time under pressure causes tissue damage in the root elongation area and death of plants.

Thus, significant, but not lethal increasing of atmospheric pressure can be considered as a stress signal causing changes in the intercellular water transport in plants.

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Mg-dechelatase initiates chlorophyll degradation and controls the gene expression of chlorophyll metabolism

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Chlorophylls play essential roles in photosynthesis such as harvesting light energy, transferring excited energy and driving electron transport. In green plants, two different chlorophyll species, chlorophyll *a* and chlorophyll *b*, are involved in these processes.

The chlorophyll metabolic pathway has long been studied and a major metabolic pathway has been elucidated in green plants [1, 2]. According to our current knowledge, chlorophyll *a* is synthesized from 5-amino levulinic acid through multiple steps. At the last step of chlorophyll synthesis, a portion of chlorophyll *a* is converted to chlorophyll *b* by chlorophyllide *a* oxygenase via 7-hydroxymethyl chlorophyll *a*. During greening, newly synthesized chlorophyll *a* and chlorophyll *b* are used for the formation of chlorophyll-protein complexes of photosystem I and II. During senescence, chlorophyll *b* must be converted to chlorophyll *a* by the chlorophyll cycle [3] before degradation. Chlorophyll *a* is then converted to pheophytin *a* by Mg-dechelatase (MgDCHEL) which extracts Mg from chlorophyll *a*. Pheophytin *a* is converted to pheophorbide *a* by pheophytinase (PPH). Pheophorbide *a* is then oxidatively ring opened to red chlorophyll catabolite by pheophorbide *a* oxygenase (PaO) followed by the reduction to fluorescent chlorophyll catabolite by red chlorophyll catabolite reductase (RCCR) [2]. All the enzymes responsible for the major chlorophyll metabolism have been identified except MgDCHEL.

Expression of the genes responsible for the chlorophyll metabolism is strictly regulated depending on the environmental conditions and developmental stages. All the identified chlorophyll degradation enzymes are induced during senescence. Interestingly, when one of the chlorophyll degradation enzymes such as PPH, PaO and RCCR is mutated and lost its function, chlorophyll degradation is delayed and the mutants show stay green phenotype, although the enzyme does not catalyze the first step of chlorophyll degradation. This indicates that some mechanisms must exist for the coordinated regulation of chlorophyll degradation enzymes.

Recently, we succeeded to identify MgDCHEL which catalyzes the first step of chlorophyll degradation. When MgDCHEL is transiently expressed in fully green leaves of *Arabidopsis thaliana* by using Dexamethasone system, almost all chlorophyll was degraded in two days. The intermediate molecules of chlorophyll degradation pathway were not accumulated indicating that other chlorophyll degradation enzymes are also activated by the expression of MgDCHEL. Microarray analysis showed that genes for chlorophyll biosynthesis were down regulated but those for chlorophyll degradation were up-regulated by the expression of MgDCHEL. Considering that all the genes responsible for chlorophyll metabolism are encoded in nuclear genome, some chloroplast signal might be produced by the expression of MgDCHEL and the signal control the expression of nucleus gene. In this conference, I will discuss the chloroplast signal produced by MgDCHEL in relation to the coordinated regulation of chlorophyll degradation pathway.

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Chloramphenicol mediated superoxide production via Photosystem I and Photosystem II in isolated spinach thylakoid membranes

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We characterized the effect of chloramphenicol on the rate of photodamage in isolated spinach thylakoid and PSII particles membrane. Photoinhibition is the light induced impairment of photosynthetic activity, which arises from the imbalance of photodamage and protein synthesis dependent repair of the Photosystem II complex. These two processes can be separated from each other by protein synthesis inhibitors, such as the frequently used chloramphenicol or lincomycin. Although these inhibitors are not expected to induce any additional damage of Photosystem II activity, previous data indicate that this might not be the case for chloramphenicol. In this study we show that light induced loss of Photosystem II activity is enhanced in the presence of chloramphenicol both in thylakoids and BBY membranes particles. We have observed that chloramphenicol induced light dependent O_2 consumption both in isolated thylakoid and BBY membranes particles. This effect was partly reversed by the addition of superoxide dismutase, which provided protection against the chloramphenicol induced photodamage. The results show that chloramphenicol, which is used as protein synthesis inhibitor in photoinhibition studies, accelerates photodamage in isolated thylakoid and PSII membrane particles. Altogether, it is concluded that chloramphenicol accelerates photodamage via superoxide production both in PSII and PSI, which causes an artifact. Therefore, the application of chloramphenicol for determination of the rate of photodamage in photoinhibitory studies should be avoided. In this study a comprehensive picture describing how chloramphenicol affects PSII photoinhibition and involves in superoxide production in isolated spinach thylakoid and BBY membranes will be explained and discussed.

Occurrence of state transitions in arabidopsis and barley plants under various illumination

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The process of state transitions is an important photosynthetic response to changes in the light composition that optimizes the energy distribution between photosystems. In the present study we gain insight into the regulation of state transitions in various higher plants at different light intensities. Representatives of the two classes of plants were used in the work, monocotyledon *Hordeum vulgare* (barley) and dicotyledon *Arabidopsis thaliana*, which are characterized by different sensitivity to the light conditions.

At first, the methodological approach, which allows estimating the occurrence of state transitions in whole leaf, has been developed. It is based on the measurements of the relaxation kinetics of non-photochemical quenching (NPQ) after short-term illumination, using Arabidopsis wild type vs. *stn7* knockout plants as well as barley leaves, which have been treated by NaF to inhibit phospotase.

Knockout of STN7 kinase leads to the lack of the state transitions occurrence under illumination and accordingly the lack of NPQ recovery part, which reflects state trasitions. Usage of NaF leads to the lack of the state trasitions-related NPQ due to blocking of the state transitions recovery in the dark. It has been established that the duration of illumination by actinic light gives the main impact on the reliability of the state transitions estimation.

Further, based on the established approach, the regulation of state transitions in arabidopsis and barley plants under various illumination has been investigated. It has been found that the different intensity of the actinic light has a different impact on the occurrence of state transitions in these plants. In arabidopsis plants, the state transitions are inhibited by the increase in the light intensities higher than 300 μmol quanta m^{-2} s^{-1}, while in barley plants state transitions occur till higher intensities, 800 μmol quanta m^{-2} s^{-1}.

The data obtained using NPQ relaxation characteristics measured by the chlorophyll *a* fluorescence at room temperature were supported by the measurements of the chlorophyll fluorescence at low temperature.

The measurements of the low-temperature chlorophyll fluorescence revealed that at high intensity of actinic light, higher than 1200 μmol quanta m^{-2} s^{-1}, the drastic changes in the ratio of peaks of photosystems fluorescence occurred. These changes implied a strong rearrangement of the photosystem II and photosystem I that does not relate to the state transitions occurrence. In wild type and stn7 knockout plants the same significant changes in the ratio of the peaks have been observed, confirming the above conclusion.

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Partial loss of the cytochrome c by mitochondria of pea seedlings under conditions of water deficit

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The mitochondria are the "energy powerhouses" of cells, play a major role in the body's response to the action of stress factors (Grabelnykh O.I., 2005). Stress factors causes a shift of antioxidant–prooxidant balance towards increased production of ROS that results in the development of a number of pathological conditions, caused by damage of cell components (Tailor N.L. et al., 2003). Found that the damaging effects of reactive oxygen species on the respiratory activity are linked to their action on cardiolipin, which is essential for the effective functioning of the respiratory chain of mitochondria. In addition, lipid peroxidation of membranes and, especially cardiolipin, leads to oxidation of the thiol groups of proteins to swelling of mitochondria and release of cytochrome C. The release of cytochrome C from mitochondria can not only be a triggering factor of apoptosis, but on the contrary, the result of the action on mitochondria factors causing apoptosis.

In this regard, the aim of this work was to investigate the effect of two days of water deficiency on bioenergetic characteristics of mitochondria of 6-day etiolated pea seedlings (*Pisum sativum* L.) cv. Alpha.

The water deficit led to LPO activation in the mitochondrial membranes of pea seedlings. In this case, the fluorescence intensity of LPO products increased 3 times. LPO activation caused significant changes in the content of the C_{18} and C_{20} fatty acids (FA) in the mitochondrial membranes. At the same time there was a decrease of the relation in the total content of C_{18} unsaturated FAs to the content of stearic acid from 16.61 ± 0.30 to 10.59 ± 0.20 and the ratio (20:1ω7 + 20:1ω9 + 20:2ω6)/C20:0 in mitochondrial membrane lipids decreased from 3.65 ± 0.03 to 1.20 ± 0.16.

Changes in physico-chemical properties of mitochondrial membranes was accompanied by a 30% decrease in maximum rates of oxidation of NAD-dependent substrates and a 25% reduction in the efficiency of oxidative phosphorylation. The dysfunction of complex I of the electron transport chain of mitochondria, apparently connected with the oxidation of unsaturated fatty acids included in the composition of cardiolipin, mainly linoleic acid (Paradies G. et al., 2004). Confirmation of this hypothesis is a 2-fold reduction in the rates of electron transport on the terminal stage of the respiratory chain of the pea seedlings mitochondria in situations of water scarcity. Introduction to the incubation medium of these mitochondria, 5×10^{-6}M of cytochrome C led to the recovery speed of oxidation of the pair ascorbate + TMPD to control values, indicating a loss of part of the mitochondrial cytochrome C due to the oxidation of cardiolipin in the inner membrane of these organelles.

It is known that cytochrome C in mitochondria, performs several functions. Firstly cytochrome C has the ability to catalyze peroxidation of cardiolipin. While the oxidized cardiolipin loses its affinity for cytochrome C, which can leave the intermembrane space of mitochondria after permeabilization of its outer membrane (Kagan et al., 2005). Secondly, cytochrome C can also obtain the electron in the process of neutralization of the superoxide anion radical, herewith there is regeneration of molecular oxygen (Korshunov et al., 1999). In the third , cytochrome C, in structure of the cytochrome C peroxidase, capable of decomposing hydrogen peroxide for account of oxidation of enzyme heme by peroxide and regeneration heme by electrons, which it received from cytochrome C (Yonetani,Ray, 1965). In this regard, it can be concluded that cytochrome C is a key switch between utilization of ROS and its generation in the mitochondria. It is assumed that the signaling function of cytochrome C serves to homeostasis in the level of ROS in the mitochondria (Díaz-Moreno et al., 2011). In this case, the loss of cytochrome C by mitochondria, may be associated with changes in the functional state of mitochondria pea seedlings.

SESSION 4

Stress-Induced Signalling

pH Measurement in root cells using Pt-GFP expressed in *Arabidopsis thaliana* (L.)

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Regulation and stability of pH of the cytosol and intracellular compartments are essential for plant cell vital activity because all metabolic processes, protein structure, ion channels activity and membrane throughput depend on the acidity. However, most of the pH registration methods enable to estimate in vivo only extracellular acidity. To solve such problem GFP-based proteins (Green Fluorescent Protein) integrated into plant genome and expressed in cell enabling intracellular pH measurement *in vivo* may be used.
The aim of this work is evaluation of *Arabidopsis thaliana* (L.) intracellular pH using laser scanning microscopy with Pt-GFP as ratiometric pH-sensor.
Studies were carried out on transgenic *A. thaliana* plants expressed pH-sensor Pt-GFP (Notthingham Arabidopsis Stock Centre, UK) and control *A. thaliana* plants (ecotype Columbia) grown *in vitro* on Murashige-Scoog medium.
Fluorescent images of control and transgenic plant roots were obtained using of Confocal laser scanning microscopy (LSM 710 Carl Zeiss, Germany). The excitation wavelengths were set to 405 and 488 nm and the fluorescence emission window was set to 500 to 512 nm. The fluorescence ratios F405/F488 were obtained as a measurement of pH on a pixel-by-pixel basis. The calibration of fluorescence intensity ratio dependence on intracellular pH in pH range of 5-8 units was made with 6 day seedlings which were placed in buffer solutions. The Pt-GFP ratio signal of root cells was calibrated using sodium citrate ($5 \leq pH \leq 5.5$; 50 mM), MES ($6 \leq pH \leq 7$; 50 mM), HEPES ($7.5 \leq pH \leq 8.0$; 50 mM).
For fitting sigmoidal curves to calibration data the Boltzmann fit has been chosen. Fitting has been performed using Origin 8 (OriginLab Corp., Northhampton, MA, USA).
Subsequently we used Pt-GFP for study of Arabidopsis roots intracellular pH. Now we apply this method for investigation of different factors (temperature, salinization, drought) influence of plant organisms.

Signalling alteration is the main reason for stomata malfunctioning in low VPD-exposed plants

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Growing or long-term exposing plants to low VPD reduces leaf capacity to maintain adequate water status thereafter. To find whether morphological and anatomical changes are the main cause of stomatal malfunctioning or signalling alterations, two experiments were conducted on the plants that exposed to different VPDs. In the first experiment to investigate the impact of stomatal morphology and leaf anatomy, on functioning of stomata, fava bean plants were grown at low (L, 0.23 kPa) or moderate (M, 1.17 kPa) VPDs and some plants that developed their leaves at moderate VPD were then transferred for four days to low VPD (M→L). Part of the M→L-plants were sprayed with ABA during exposure to L. L-plants showed bigger stomata, larger pore area, thinner leaves and less spongy cells compared with M-plants. Stomatal morphology (except aperture) and leaf anatomy of the M→L-plants were almost similar to the M- plants, while their transpiration rate and stomatal conductance were identical to that of L-plants. The stomatal response to ABA was lost in L-plants, but also after 1-day exposure of M-plants to

low VPD. The level of foliar ABA sharply decreased within 1-day exposure to L. Spraying ABA during the exposure to L prevented loss of stomatal closing response thereafter. The effect of low VPD was largely depending on exposure time: the stomatal responsiveness to ABA was lost after 1-day exposure to low VPD, while the responsiveness to desiccation was gradually lost during 4-days exposure to low VPD. Leaf anatomical and stomatal morphological alterations due to low VPD are not the main cause of loss of stomatal closure response to closing stimuli, but can strengthen the effect of VPD on stomatal behaviour when plants have developed their leaves at low VPD.

Polyamines alter cyclic guanosine 3',5'-monophosphate levels in *Arabidopsis* roots under salt stress

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Soil salinization is a significant factor that limits crop productivity worldwide. A comprehensive understanding of biophysical mechanisms of stress-signal transduction pathways is an urgent challenge of modern biology and could be useful in the development of new techniques for enhancing the resistance and productivity of agricultural crops. Cyclic guanosine 3',5'-monophosphate (cGMP) is a well-established intracellular molecule involved in diverse physiological processes, biotic and abiotic stress responses in higher plants. Salt stress was found to induce cGMP response in *Arabidopsis* seedlings. It is known that one of the protective mechanisms in response to abiotic stresses is accumulation of low molecular weight organic antioxidants (polyamines, proline, phenols, sugars, etc.) in plant cells, which reduce the damaging effect of the stress factors. However, the interplay between polyamines and cGMP-dependent signalling system in response to salt stress has not been established in plants so far.

Our results showed that salt stress induces a rapid and dramatic increase in cGMP levels in *Arabidopsis* roots. Increases in cGMP levels were detectable within 10 sec after treatment with 100 mM NaCl reaching a maximum within 1 min (4.6-fold increase in comparison to untreated seedlings) and decreasing to the prestimulation level in 10 min. Polyamines (1 mM spermine, spermidine and putrescine) were shown to stimulate significant increase in cGMP concentration in *Arabidopsis* roots after 30 sec of incubation reaching a maximum within 1 min and returned to background levels within 15 min. Spermidine was found to induce the maximum amplitude of cGMP response (2.8-fold increase in comparison to untreated seedlings).

It was shown that pretreatment of *Arabidopsis* roots with 1 mM spermidine reduced the amplitude of cGMP response after 100 mM NaCl treatment suggesting that the effects of spermidine under salt stress are implemented with the involvement of cGMP-dependent signalling pathway in plants.

In literature polyamine-induced nitric oxide (NO) release was demonstrated in *Arabidopsis* with no apparent lag phase. It is well known that NO may act through the activation of guanylate cyclase. Previously we have shown that NO (using the NO donor sodium nitroprusside (SNP)) activated guanylate cyclase in *Arabidopsis* seedlings and stimulated significant increase in cGMP levels within 1 min.

To study the interaction between NO and cGMP in response to spermidine *Arabidopsis* roots were preincubated with the NO scavenger 1 mM 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt (cPTIO) for 1 h before spermidine treatment. Pretreatment of roots with cPTIO entirely suppressed spermidine-induced cGMP response. Application of cPTIO with spermidine as pretreatments completely abolished the effects of spermidine on cGMP release under salt stress.

Summing up, our data suggest that under salt stress polyamines act through cGMP-dependent signalling pathway with the involvement of NO.

Toward the identification of a partnership belonging to a multi-step phosphorelay system as signaling pathway in poplar drought perception

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The molecular basis of plant drought perception and the resulting cellular osmotic stress are still unknown. In yeast, the HOG pathway controlled by the multi-step phosphorelay system (MSP) SLN1-YPD1-SSK1, is the best characterized osmosensing pathway, leading to yeast adaptation on high-osmolarity and high-salt media due to glycerol accumulation. In Populus, we identified MSP proteins such as a Histidine-aspartate Kinase, HK1, homologous to SLN1, Histidine- containing Phosphotransfer proteins, HPt1 to HPt10, and 9 Response Regulators (RR). By combining several approaches including yeast two-hybrid and bimolecular fluorescence complementation assays and co-expression analysis of mRNAs encoding studied proteins, we showed the existence of a partner network linked to HK1, the putative poplar osmosensor. This network involves HK1, HPt2, 7 and 9 and six transcription factors belonging to the RR-B family, RR12, 13, 14, 16, 18 and 19 (Chefdor et al., 2006, Héricourt et al., 2013, Bertheau et al., 2012 ; 2013 ; 2015).
Even if the "osmosensor" function of HK proteins such as AHK1 in Arabidopsis is still not clearly attested, a role in dehydration avoidance is well established now (Kumar et al., 2012). Thus, in poplar, the HK1 MSP could be involved in dehydration-sensing mechanisms and plant water status control. To go further in this study and better understand the role of this HK1 MSP in drought tolerance, we are now investigating the HK1 function by a loss of function approach based on RNAi strategy.

Glycation of plant proteins under stress conditions

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Protein glycation is a non-enzymatic post-translational modification formed by interaction of free protein amino groups with carbonyl compounds (carbohydrates and α-dicarbonyls). In the first step, reducing sugars (aldoses and ketoses) interract with lysyl residues, yielding Amadori and Heyns compounds, respectively. These early glycation products are readily involved in degradation, rearrangement, and cross-linking reactions yielding a heterogeneous group of advanced glycation end-products (AGEs). Alternatively, α-dicarbonyl intermediates of monosaccharide autoxidation can be precursors of these compounds. Formation of AGEs was demonstrated during thermal processing of foods and *in vivo* in mammalian tissues. Thus, in mammals, AGEs are known for their toxic and pro-inflammatory effects. Recently, this modification was shown to be common in plants as well. Due to simultaneous increase of tissue sugar contents and reactive oxygen species (ROS) production under environmental stress, glycative and glycoxidative damage of plant proteins might be characteristic for

plant stress response. In this context, it is important to characterize the changes in AGE-modified plant proteome under environmental stress conditions, as well as metabolic pathways underlying AGE formation and related defense mechanisms. To address these questions the models of high light, drought and cadmium metal stress were established with *Arabidopsis thaliana* and *Brassica napus*. The leaves were harvested throughout the stress period in parallel to comprehensive stress characterization with a panel of reliable markers. Proteomic and metabolomic analysis relied on LC- and GC-MS analysis, respectively. All stressors caused development oxidative stress, and triggered significant carbohydrate accumulation in treated plants. As was revealed by *in vitro* experiments, many of the accumulated sugars were highly-reactive towards synthetic peptides. Surprisingly, *in vivo* overproduction of carbohydrates was not accompanied with elevation of free α-dicarbonyl levels. It might indicate their binding to cellular proteins and metabolites directly after their generation. Moreover, environmental stress resulted in characteristic patterns of AGEs dominated with arginine-derived modifications originated from α-dicarbonyls. Remarkably, formation of lysine-derived AGEs did not affect the sites of early glycation. This might indicate that degradation of Amadori/Heyns products does not contribute significantly in AGE formation. Taken together the results suggest that the changes in AGE patterns depend on the nature of environmental stressor. Nevertheless each stressor enhanced advanced glycation most probably via increase of monosaccharide autoxidation levels.

Extracellular biologically active peptides in wheat cell culture under stress condition

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The purpose of this research was to isolate fractions of extracellular peptides from wheat cell suspension cultures grown under the salt stress conditions (0,1%, 0,25%, 0,5%, 0,75%, 1,0%, 1,68% NaCl) and to study their growth-regulating and protective physiological activities.

In the one-step assay it was revealed that extracellular peptides' fractions obtained under different NaCl concentrations were different in their qualitative composition of chromatographic profiles determined by reversed phase high performance liquid chromatography (RPh-HPLC) in variants with a maximum (1.68 %) and lower concentrations (0, 1-1,0%) of NaCl compared each other and control (no salt). Mass-spectrometric analysis of the fractions collected after RPh-HPLC allowed us to establish that they have molecular weights in the range of peptides (2,0-8,1 kDa).

Additionally, for the improvement of the quality of components separation we have been using a two-stage method of analysis based on the combination of liquid chromatography methods on molecular weight and hydrophoby. Thus, exclusion chromatography of extracellular liquids' total extract in control and variants with 0.5 % and 0.75 NaCl allowed us to collect the prevailing fractions, which, according to mass-spectrometry, are components with masses ranging 5,0-7,5 kDa. These fractions were rechromatographed by RPh–HPLC method. When comparing the profiles of variants on the number of components and retention time, there were no differences between control and variant with 0,75% NaCl, whereas the spectrum of components in variant with 0,5% NaCl was different from control.

The study of biological activity of extracellular peptide compounds from variants with 0,5% NaCl, corresponding to weight in the range of 5,0-7.5 kDa and separated into fractions by RPh-HPLC on 9 peaks, has revealed their high physiological activity, which showed an increasing the survival of the test plants under the salt stress. Investigated fractions stimulated percent and energy of germination of mustard seeds, growth of roots and hypocotyls, increase in chlorophyll content and biomass accumulation of mustard cotyledons under the stress generated by sub-lethal dose of NaCI 0,75%, compared to control (0,75% NaCI, without adding peptide fractions).

It is important to point out that biological activity of fractions in conditions of salt stress was shown in the nano- and picomolar concentrations, and, in some cases, had saltatory character. Both of these features are characteristic of signaling molecules.

Thus, we have identified the extracellular fractions of biologically active peptides released by cultured plant cells in response to salt stress, which can act as signaling molecules that stimulate growth and protective processes under stress at the cellular level and level of whole plants.

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Plant G proteins come of age: Breaking the signaling bond with animal models

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Heterotrimeric G-proteins (G-proteins), consisting of α, β and γ subunits, are universal signal transducing proteins that, in animals, mediate signaling from G-protein coupled receptors (GPCRs). G-proteins have been known for a long time and most research has been concentrated in humans where they play crucial roles in a multitude of cellular and developmental pathways[1]. The attention that G-proteins have attracted from scientists can be easily stated by numbers: since Alfred Gilman and Martin Rodbell received the Nobel prize in 1994 for their discovery[2], there have been in excess of twenty two thousand peer-reviewed publications dealing with G-proteins or their associated GPCRs, of which, only an infinitesimal part are devoted to plants (<350). In view of the vast amount of knowledge accumulated in animal systems it is not surprising that from the very beginning plant G proteins have been modelled on their animal counterparts and, most importantly, studied as an extension of the animal paradigm.
Although nobody dared to openly admit it, and thus confront the animal research 'big brothers', it was clear from the very beginning that plant G-proteins had developed some 'peculiarities' openly differentiating them from their animal counterparts[3]. For example, while humans contain multiple subunits (a combined total of 40); Arabidopsis was originally restricted to 1 Gα, 1 Gβ and 2 Gγ subunits. With time, our group and others discovered additional and highly divergent Gα subunits (more than double the size of the canonical one) and Gγ subunits containing a transmembrane domain and a large receptor-like extracellular region[4,5].
In addition to the structural differences, the biochemical properties of plant and animal G-proteins are also quite different. The hydrolysis rate of plant Gα subunits is considerably lower than the animal Gαs, to such extent that plant Gαs have been proposed to be "constitutively active"[6]. The existence of plant GPCRs is also hotly debated and we will present evidence that, in plants, G-proteins mediate receptor like kinase (RLK) signalling and physically interact with a number of RLK.
We firmly believe that the accumulated body of evidence as well as our unpublished results prove that plant and animals have followed different 'G protein evolutionary paths'. We will discuss the 'long and winding road' that plant G proteins have taken on their way through puberty and finally independence from their 'animal relatives'. Most importantly we will present a hypothetical model for plant G-proteins that does not rely on GTP/GDP exchange for activation.

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Identification and analysis of Triticea mitogen-activated protein kinases

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Triticea is a tribe of Poaceae species which includes major grain crops grown around the world such as wheat and barley. Despite their socioeconomic importance, very little research in the area of mitogen-activated protein kinase (MAPK) signaling, which is central to regulation of most plant behavior, has been reported in these crops. The MAPK family is divided into three major subfamilies: the MAPK kinase kinases (MAPKKKs) that activate members of the second subfamily, MAPK kinases (MKKs), by phosphorylation; these, in turn, phosphorylate the third subfamily of MAP kinases (MPKs). Once activated, MPKs regulate a variety of targets, including numerous transcription factors and enzymes, through phosphorylation. The goal of this study was to identify MAPKs within the Triticea tribe and to establish their nomenclature in wheat, barley, rye and triticale (a wheat x rye hybrid). Phylogenetically, the plant MAPKKKs are categorized in three subgroups (MEKK, ZIK and RAF), and the MKK and MPK subfamilies are each divided into four groups (A, B, C and D). As in the prototypical mammalian MAPK (ERK), the A, B, and C subgroups of the plant MPK subfamily typically have a conserved TEY motif in their activation loop. Members of the D group, which are exclusive to plant species, are distinct, with a TDY motif. Interestingly, a departure from the usual TEY motif in group B was observed in the Triticea MPK11s, which were consistently shown to possess an MEY motif; this motif has also been identified in some MPKs outside Triticea members, including rice and tomato. We identified another novel motif in the activation loop of a group B member, where one genomic copy of wheat MPK7-2 was shown to possess a TGY activation loop motif. Consistent with the complexity of the polyploid genomes of wheat and triticale, multiple copies of many MAPKs were identified, and phylogenetic analysis revealed a particularly notable expansion of the group D MPKs and MKKs. To our knowledge, the TGY motif has not yet been reported in plants, but it is present in the mammalian p38 MPK activation loop. A report of p38-like activity in wheat indicates the potential functional significance of such MAP kinases in plants. Protein interaction patterns between members of the wheat MAPK family, together with tissue-specific developmental gene expression and enzyme activation patterns have provided insight into the functional significance of MAPKs in the regulation of myriad metabolic processes. The data presented here expand our existing knowledge of the structure of MAPK families in plants, as well as providing novel insights specifically for Triticea species. Furthermore, this study sets the stage for more advanced signal transduction research in these important grain crops.

Possible components of sodium sensing pathway in plants

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Changes in the concentration of external Na^+ ions can affect plants in a variety of ways. High external Na^+ is detrimental to the majority of plants; moderate Na^+ concentrations cause salt acclimation without stressing the plant; low concentrations of Na^+ stimulate plant growth under certain conditions. To optimize their growth and development, plantsneed to constantly monitor Na^+ concentration in soil solution. The plant molecular mechanisms responsible for sensing external Na^+ concentrations remain largely unknown. Mainly because of the damage caused by salinity on agriculture, vast resources have been spent to unravel the tolerance mechanisms that plants employ to counter salt stress. Salt stress causes significant changes not only to soil osmotic status, but also to soil concentration of potentially toxic Na^+ and Cl^- ions, all of which is likely to affect many plant signaling pathways. The multitude of effects of salt stress make it very challenging to use it to unravel plant molecular mechanisms specifically recognizing changes in extracellular Na^+ concentration. Treating Arabidopsis with solutions with low Na^+ concentrations and employing K^+ treatment as a control, we have separated osmotic, Na^+ and Cl^- components of the salt treatment. Our data indicate that change in external Na^+ concentration from 1 to 4 mM induces rapid changes in extracellular proton and reactive oxygen species concentrations in Arabidopsis root, and induces long-lasting changes in Arabidopsis root and shoot proteome. These

results suggest that Arabidopsis plants sense small changes in the extracellular concentration of Na^+ and modify their proteome in response.

Lipoxygenase signaling system in plant growth and developmental processes

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The oxidative metabolism of polyenoic fatty acids through the lipoxygenase pathway is a source of numerous oxylipins, which play important roles in regulation of plant growth and development, cell signaling and defense. Metabolism of fatty acid hydroperoxides and thus the diversity of oxylipins largely depend on the enzymes of CYP74 family. These are allene oxide synthases (AOSs), hydroperoxide lyases (HPLs), divinyl ether synthases (DESs) and epoxyalcohol synthases (EASs). These enzymes convert the fatty acids 9- and 13-hydroperoxides into bioactive compounds collectively called oxylipins (hydroxy-, oxo-, or keto-fatty acids, divinyl ethers, volatile aldehydes, jasmonates and others). The understanding of processes regulating the biosynthesis, localization and functions of these bioactive compounds in cell is crucial for understanding of the biology of this family of metabolites.
Earlier we identified novel oxylipins and enzymes involved in their biosynthesis in the different plant taxa. For the first time recombinant 13-specific DESs of *Linum usitatissimum* (LuDES), *Ranunculus acris* (RaDES) and *Selaginella moellendorffii* (SmDES1 and SmDES2) were prepared and biochemically characterized. Besides, we confirmed the presence of EAS pathway and characterized the corresponding enzymes – EASs, the presence of which was disputable for a long time. In addition, we had deciphered the scheme of the catalytic mechanism of these enzymes.
For understanding of key physiological processes, in particular, the characteristics of functioning of signaling systems, valuable information can be obtained from the data about dynamics of gene expression during ontogenesis and its changes under environmental conditions. However, not all enzymes encoded by the genes of interest have biochemically been characterized yet. Researchers are often content with annotations of enzymes based on amino acid sequence homology. This approach often gives misleading information. In our laboratory we have obtained quite a lot of enzymes with high sequence homology to enzymes possessing a completely different catalytic activity.
At present work we have cloned and biochemically characterized all CYP74 enzymes of the selected model object *Cucumis sativus*. These are one AOS (CsAOS), one EAS (CsEAS), and two dualistic enzymes possessing HPL and EAS activity. Products of conversions of linoleic and alpha-linolenic acid 9- and 13-hydroperoxides catalyzed by the recombinant CYP74 enzymes of cucumber were identified by the data of mass spectrometry, NMR and UV spectroscopy. Target gene expression evaluation showed that changes in the expression levels have not only the organ specificity, but also depend on the stage of plant development.
Experiments on the study of CsAOS and CsEAS were supported by grant 15-04-08310-a from the Russian Foundation for Basic Research (RFBR). Experiments on the study of gene expression dynamics was supported by grant MK-6529.2015.4. Experiments on the study of two dualistic enzymes possessing HPL and EAS activity was supported by the Russian Science Foundation (Project No. 16-14-10286).

Two antagonistic calmodulin like proteins modulate plant defense against insect herbivores

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Plants are attacked by a plenty of different herbivores throughout their life. A lot of them belong to arthropods, mainly to the class of insects. As the most species rich class of eukaryotes, insects show a huge variability in their feeding strategies: While some of them cause mechanical damage to the plant (e.g. chewing insects), others like piercing sucking insects cause only minor damage. To overcome these different attacks, plants developed a variety of direct and indirect defense mechanisms. Phytohormones like jasmonates coordinate these defenses. In contrast, less is known about the recognition of the feeding insect and early signal transduction linking to downstream pathways.
In response to the herbivore attack, changes of the intracellular calcium (Ca^{2+}) level are one of the first signaling events in plants. However, Ca^{2+} elevations are ubiquitous signals originating from biotic and abiotic stimuli. To react appropriately to each stimulus, specific decoding of such Ca^{2+} signals is essential for the plant. Binding of Ca^{2+} ions by calcium sensor proteins is an important first step of decoding. In *Arabidopsis,* 250 calcium binding proteins are known. Microarray data revealed that a group of them, the calmodulin-like proteins (CMLs), are regulated upon herbivory. This study is focusing on two of them: CML37 and CML42.
Both of them are calcium sensing proteins with 3 EF-Hands (calcium binding domains). Recently, it has been shown that both CMLs regulate response to the chewing insect herbivore *Spodoptera littoralis*. Whereas *CML37* is mainly induced by mechanical wounding upon feeding of the insect, *CML42* is only induced by elicitors in the oral secretion of the herbivore. Knock out of *CML37* leads to a better performance of larvae suggesting that CML37 is a positive defense regulator in *Arabidopsis*. While there are no changes in secondary metabolites like glucosinolates, the production of jasmonates and expression of JA-responsive genes is significantly reduced in loss of function mutants. In contrast, knock out of *CML42* leads to a reduced performance of larvae suggesting that CML42 is a negative defense regulator in *Arabidopsis*. The *cml42* mutants show a different constitutional level of glucosinolates as well as an increased sensitivity to jasmonates. Thus, CML37 and CML42 might act as antagonistic regulators of herbivory-induced defenses in *Arabidopsis* plants. To investigate further the antagonism of both calcium sensors double knock out mutants lines were constructed and analyzed.

Counter-directed changes in water transmembrane and symplast transport in plant roots as the features of systemic response to abiotic stresses

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The effect of stress factors on plants induces a number of defensive responses, both local in cells immediately contacting with effectors and systemic in cells distal from the treatment site. To reveal the mechanisms of plant abiotic stress adaptation, it is important to study early systemic responses concerning plant intercellular water exchange, and mobilized by cell-to-cell communications. We studied the dynamics of early changes in membrane permeability and symplast conductivity for water in intact plants under osmotic and oxidative stress. In the course of experiments the maize root tips were treated with various osmotic compounds (PEG-6000, NaCl, -0.5 MPa), hydrogen peroxide (6 mM) and using NMR method there was registered the diffusional water transfer in a root zone distal from treatment site in the time range from 0 to 4-5 hours after treatment.
The common early response to osmotic treatment was shown to be the increase in root transmembrane water transfer induced by aquaporin activity increase. This effect was reversible and was removed by

aquaporin blockers of various nature (propionic acid, mercury chloride). There was obtained evidence in favor of counter directed regulation of aquaporin mediated transmembrane water transfer in roots at different levels of oxidative stress, which was controlled by lipid peroxidation level. The application of paramagnetic complex (Gd-DTPA) eliminating the apoplast water signal, demonstrated the inhibition of the symplast water transfer under osmotic and oxidative stress. The symplast transfer changes for intact and excised roots differed.

Thus, various stress factors induce in plants early systemic non-specific responses characterized by the increase in intercellular water transfer in roots due to the increased aquaporin activity, and by the decrease in symplast water transfer intensity. The difference in root water permeability dynamics in various cultures under osmotic stress depend on both original water permeability and the ability to switch the pathways of the root radial water flow (cell-to-cell, apoplast), and also can be a criterion for the choice of the strategy of response to water deficit and plant drought tolerance.

Linking salicylic acid signalling with salinity stress tolerance in Arabidopsis: lessons from *NahG* mutant

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A salicylic acid-deficient Arabidopsis *NahG* (*Naphthalene hydroxylase G*) transgenic line is known to have enhanced salt tolerance. However, the specific ionic mechanisms mediating this tolerance remain unknown. The growth, ion content analysis, viability staining, membrane potential and ion flux (Na^+, K^+ and H^+) measurements were made to characterize *NahG* and wild type plant responses to salinity and oxidative stresses. The growth and viability staining results suggested that *NahG* was salt tolerant but oxidative stress sensitive. Salt stress decreased K^+ concentration in the shoot, but no significant difference was found between *NahG* and WT plants. By contrast, *NahG* had higher shoot Na^+ concentration but lower Na^+ influx into the roots compared with wild type. Interestingly, *NahG* showed higher salt-induced K^+ loss than wild type, but this K^+ loss was not related to stress-induced membrane depolarization and activity of depolarisation-activated K^+ outward-rectifying channels. Instead, higher K^+ loss in *NahG* was mediated by ROS-activated non-selective cation channels. *NahG* also had lower net H^+ influx in the mature root zone, both under control and stress conditions, presumably due to increased H^+-ATPase activity. This was consistent with observed 10 mV difference (more negative in *NahG* than WT plants) in membrane potential of root epidermal cells. It is concluded that salt tolerance of *NahG* is not related to prevention of salt-induced K^+ loss. Instead, *NahG* exerts better control over initial Na^+ uptake followed by preferential Na^+ transport and vacuolar sequestration in shoots during salt stress.

The role of calcium-dependent protein kinase CPK26 gene in the resistance of wild-growing grapevine Vitis amurensis Rupr. to abiotic stress conditions

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Abiotic stresses, such as extreme temperatures, soil salinity, or water deficit, cause extensive losses to agricultural production worldwide. Examination of molecular and genetic mechanisms of abiotic stress tolerance in plants is of great interest to plant biologists. Recent advances have revealed calcium-dependent protein kinases (CDPKs or CPKs), which are the most important Ca^{2+} sensors in plants, play crucial role in plant adaptation to abiotic stress (Asano et al., 2012a; Schulz et al., 2013; Boudsocq and Sheen, 2013). We focused on studying the roles of *VaCPK26* gene in the adaptation to abiotic stress of wild-growing grapevine *Vitis amurensis* Rupr., which is known to posses high level of resistance against adverse environmental conditions. Earlier, we studied *VaCPK26* expression under different abiotic stress treatments using healthy *V. amurensis* cuttings. Real-time PCR revealed that mRNA level of *VaCPK26* was significantly up-regulated under salt and cold stresses (Dubrovina et al., 2013 J Plant Physiol).

In this study, we obtained four transgenic cell lines of *V. amurensis* overexpressing the *VaCPK26* gene and a control cell culture transformed with the «empty» vector (it contains only *nptII* gene in the T-DNA) using *Agrobacterium*-mediated transformation. We examined the effect of salinity, heat, mannitol and cold stresses on growth of the four *VaCPK26*-transgenic *V. amurensis* cell cultures. In addition, using floral-dip transformation method (Zhang et al., 2006), we transformed *Arabidopsis thaliana* with the *VaCPK26* gene *in planta* and obtained four transgenic plant lines overexpressing *VaCPK26*. The transgenic plants were subjected to salinity, heat, cold and drought stress treatments, thereafter the survival rates of the four *VaCPK26*-overexpressing *Arabidopsis* lines were determined. The data show that the growth of all *VaCPK26*-transgenic *V. amurensis* cell lines under salt stress was better than the growth of the control cell line. The analysis of the *V. amurensis* cell lines tolerance to heat, mannitol and cold stresses revealed that the growth of the vector control did not considerably differ from the growth of 35S-*VaCPK26* transgenic cell lines. The two lines of *VaCPK26*-overexpressing *Arabidopsis* out of the four tested were tolerant to salt stress compared to control plants, however only in two experiments. Therefore more experiments are needed to verify these results. Heat, cold and drought stress resistance levels of the transgenic *A. thaliana* were comparable to that of the controls. The results obtained suggest that the *VaCPK26* gene is involved in salt stress adaptation of the grapevine.

Signalling in response to changes in temperature in Arabidopsis

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We are taking a combined approach to determine signalling pathways which lead from perception of cold, through activation of cold acclimation in order to finally induce the mechanisms required for tolerance to freezing. Our work has identified the importance of calcium in low temperature signalling leading to the regulation of cold gene expression through calcium regulated transcription and factors. We have used empirical and mathematical modelling approaches to characterise the low temperature calcium signal and, additionally, we have investigated the role of calcium in mediating appropriate patterns of stress gene expression. For this we have taken a transcriptomic approach to define the specific transcription factor systems that are regulated by calcium and a mathematical approach to begin to understand how calcium encodes information that specifies the correct pattern of gene expression. In parallel we have been taking a classical genetic approach to identify components required for freezing tolerance: namely the identification of mutations in Arabidopsis that affect freezing tolerance. We have recently identified three new genes through a combination of mapping and next generation sequencing. Our results have uncovered roles for vacuolar ion transport, cell wall remodelling and regulation of the chloroplast genome in freezing tolerance. We are now turning our attention to the chloroplast, and investigating calcium signalling in this organelle. We have found evidence of chloroplast-specific calcium responses in response to heating, and characterised the properties of the chloroplast thermometer. These approaches, the findings obtained and the implications of these findings will be discussed in the presentation.

Changes in the content of dehydrins and stress proteins in the needles of *Pinus sylvestris* L.*, Picea obovata* Ledeb.*, Larix sibirica* Ledeb. and *Larix gmelinii* Rupr. throughout vegetation period

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Stress proteins (heat shock proteins, Hsp) accumulation in the cells is provoked by various external factors (high and low temperature, osmotic stress, etc.). In this process the resistance to external factors, named induced tolerance, is formed. Hsp protect intra-cellular proteins from denaturation, prevent their aggregation and provide restoration of damaged polypeptides under unfavorable external influence. Dehydrins (dhn) are a particular group of proteins protecting membranes and polypeptides under osmotic stresses which were provoked by the influences of cold, salt or drought. Adaptive dehydration during preparation for winter is one of the factors resulting in dhn accumulation in perennial plants. Seasonal dynamics of the content of Hsp 101, 70, 60, 17,6 and dhn accounted for by

necessary of seasonal adaptations was previously established for the needles of *Pinus sylvestris* (*P.s.*). The present research was designed to compare seasonal dynamics of dhn and Hsp during the vegetation period of conifers belonging to diverse ecological classes: xerophytes (*P.s.*) and mesophytes (*Picea obovata, P.o.*), as well as of deciduous conifers with different natural environment. So, *Larix sibirica* (*L.s.*) grows in warm drained ecotopes, while the areal of *Larix gmelinii* (*L.g.*) almost fully coincides with the zone of continuous permafrost. The study of 2012 was based on the needles of *L.s.*and *L.g.* brachyblasts of the second year and the second year needles of *P.s.* and *P.o.* of 30-year old trees from the experimental plantation of the institute, with further extraction of total protein. Hsp and dhn were identified by Western-blot with using of the primary antibodies against dhn, Hsp101 and Hsp 17,6 (Agrisera). Climatic conditions of 2012 favored vegetation of conifers: the level of solar radiation insignificantly differed from average-multiannual values; average monthly air temperature was higher than average-multiannual values; average monthly soil temperature at the depth of 20 cm was lower than average-multiannual values due to high humidity level throughout the whole vegetation period. In April, May and July of 2012 overall precipitation significantly exceeded average-multiannual values of this period with optimal level in other months. Hsp17.6 and Hsp101 in *P.o.* and Hsp17.6 in *L.s.* and *L.g.* were identified for the first time to our knowledge. No differences were found in accumulation of these proteins in the needles of trees belonging to different ecological categories (*P.s., P.o.*), nor any pronounced relation between accumulation of these proteins in needles and the ability for overwintering. However, the character of Hsp17.6 accumulation for *L.g.* differed from that for other trees. Different dynamics of changes in the content of Hsp17.6 in *L.g.* is likely to be connected with the ability of this tree to grow in permafrost environment. In the needles of *P.o., L.s.* and *L.g.* for the first time there were found dhn differing in mass from dhn of *P.s.*, maximum accumulation of which fell on colder months, as well as in wintering needles of *P.s.* In *P.o.* for the first time there was identified accumulation of dhn with the masses of 67, 58, 46, 42 and 11 kDa in April and October (here and hereinafter the masses are provided by electrophoresis). Dhn of *P.o.* differ from dhn of *P.s.* by their molecular masses. The variety of dhn in the needles of *P.o.* is smaller than in the needles of *P.s.*, however, seasonal dynamics of dhn coincides in both species. It is remarkable that in both *P.o.* and *P.s.* low-molecular dhn with masses 13 and 11 kDa are found only in cold period of the year. In the needles of *L.s.* and *L.g.* there were found major dhn with similar approximate molecular masses 60, 51, 47, 42 and 33 kDa. In *L.s.* dhn content in the needles was consistently high, *L.g.* dhn content was lower and significantly changed with the change of seasons. Increased dhn content was found in the needles of both trees in May and September. It is worth noting that none of larch species contained low-molecular dhn, which allows us to presume relation between accumulation of these proteins and wintering state of needles. Thus seasonal dynamics of dhn and Hsp and composition of dhn in the needles of *P.s., P.o., L.s.* and *L.g.* are infuenced by adaptation to the different environmental conditions and ability for overwintering.

Interaction between heat and light in chloroplast calcium signalling

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Calcium signals are core regulators of a wide range of plant responses to biotic and abiotic stresses as well as key triggers of several developmental processes. Whilst the function of cytosolic calcium has been widely investigated, calcium's involvement in organellar signalling, and specifically in the chloroplast is still an emerging field.

In this study we examined the role of calcium signalling in the chloroplast, specifically in response to heat. Using transgenic *Arabidopsis thaliana* seedlings in which the calcium reporter aequorin was targeted to the stroma, chloroplast-specific free calcium increases were detected in response to mild (from 32.5°C) heating. These heat-induced responses were highly reproducible, temperature-dependent and conserved amongst ecotypes (*Arabidopsis* Col and WS) and between species (*Arabidopsis thaliana*, *Nicotiana tabacum* and *Nicotiana benthamiana*). We further demonstrated that changes in membrane fluidity, through acclimation to a lower or higher temperature or overexpression of the chloroplast membrane-specific FATTY ACID DESATURASE 7, resulted into appropriately altered dynamics of the calcium increases.

An additional component interacting with this calcium signal is light: the heat-induced calcium response was delayed in the dark when preceded by a light treatment. This light-dependent delay could be partially recovered by adding the electron transport chain uncoupler DCMU. Finally, reduced calcium increases upon heating were observed in a mutant for a thylakoid membrane calcium sensor protein: CaS. The signalling difference between the *cas* mutant and wild-type were lost upon light pre-

treatment. Taken together, these data suggest that this heat-induced signalling cascade is regulated both by the presence of the CaS protein, whose function is still under investigation, and by the photosynthetic activity of the chloroplast.

Local and systemic calcium and phytohormone signaling in biotic interactions

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Plants react to both abiotic and biotic stress with an array of direct and indirect defense strategies. After the perception of a particular stress cue, an efficient and well-coordinated subsequent signaling is the prerequisite for appropriate defense reactions. Key players in such signaling processes are intracellular $[Ca^{2+}]_{cyt}$ changes as well as changes in phytohormone levels. Here, jasmonates and jasmonate-dependent pathways have an important function. Relatively little is known about the early signal transduction pathways that connect insect feeding to the plant defense responses they evoke. Although Ca^{2+} has been implicated as a second messenger in many plant signaling pathways, its specific role in herbivory is still poorly understood; however recent studies revealed that intracellular $[Ca^{2+}]_{cyt}$ elevations are decoded by Ca^{2+} sensor proteins and translated into specific cellular responses. Beside the responses in the local, treated leaf tissue, the systemic response within the plant is of particular interest. The roles of Ca^{2+} and jasmonates in the interaction between herbivores and *Arabidopsis thaliana* and their impact on downstream defense-related pathways in the whole plant are in the focus of this presentation. The underlying mechanisms of local and systemic herbivory-induced signaling will be discussed.

Does Na^+,K^+-ATPase activity modulation and distribution regulate sodium uptake in salt stressed seedlings?

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NaCl stress induced modulation of sodium uptake and its accumulation in plants involves regulation of various sodium efflux mechanisms. Earlier physiological investigations in the last two decades have reported the presence of Na^+,K^+-ATPase-like enzyme activity in plants. In this context ouabain (an inhibitor of Na^+,K^+-ATPase activity in animal systems) was used as a pharmacological probe to analyze and estimate ouabain (OU)-sensitive enzyme activity in various plant materials. These findings lead to the possibility of existence of a novel sodium efflux mechanism in plants which is functionally similar to that of Na^+,K^+-ATPase (EC 3.6.3.9) activity in animals. In continuation of these background findings, the present work demonstrates NaCl-stress (120 mM NaCl)-induced modulation and differential distribution of ouabain-sensitive ATPase activity and its correlation with sodium uptake and accumulation in sunflower seedlings using a novel fluorescent imaging approach. 9-Anthroylouabain (a fluorescent derivative of ouabain) has been used as a probe to analyze the modulation and distribution of OU-sensitive ATPase activity in the roots of sunflower (*Helianthus annuus*) seedlings subjected to 120 mM NaCl. NaCl stress from 48 h to one week elicits the induction of OU-sensitive ATPase activity in sunflower seedling roots which further shows age-dependent modulation of enzyme activity. Confocal laser scanning microscopic (CLSM) analysis further revealed NaCl stress-induced enhanced activity of ATPase in the meristematic region of 2d old seedling root tips to be associated with differential distribution of sodium ions. Furthermore, calcium-induced inhibition of OU-sensitive ATPase activity was observed in accordance with earlier reports of Na^+,K^+-ATPase inhibiton by calcium in animal systems. In the present work, calcium ions (10 mM) significantly inhibited the ATPase activity accompanied with a parallel accumulation of sodium ions in the cytosol of the meristematic cells of NaCl-stressed seedling roots. Subcellular localization of OU-sensitive ATPase activity revealed its presence in the nuclear membrane of root protoplasts and it gets inhibited after treatment with calcium ions. These findings highlight a possible correlation of ouabain-sensitive ATPase activity and regulation of sodium uptake mechanisms in NaCl-stressed sunflower seedling roots. Ouabain-sensitive ATPases in plants, therefore, appears to have its evolutionary linkage with Na^+,K^+-ATPase activity among animals both in terms of ouabain sensitivity and regulation of sodium extrusion mechanisms.

Sodium pumps in salt tolerant algae

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Many eukaryotic organisms belonging to different life kingdoms successfully survive in the environment with high NaCl concentrations. The fundamental adaptation strategy inherent for all these organisms is to prevent excessive Na^+ accumulation in cytoplasm since high Na^+ concentration in cytoplasm is toxic for cell. Under high salt environment low Na^+ concentrations in the cytoplasm are maintained due to: (1) restriction of Na^+ influx to the cytoplasm across the plasma membrane, and (2) operation of Na^+-translocating proteins localized to the plasma membrane/tonoplast and exporting Na^+ from the cytoplasm to the external medium/vacuole. In many halotolerant eukaryotes except higher plants Na^+-ATPases resided in the plasma membranes and referring to the P-type ATPase family play an important role in maintaining Na^+ homeostasis in the cytoplasm. These transport enzymes execute active sodium export from cells against steep electrochemical Na^+ gradient. Mammalian Na^+,K^+-ATPase is the first discovered and most extensively studied representative of the Na^+-transporting ATPases. More recently, Na^+-translocating ATPases of P-type, the sodium pumps, were also demonstrated in many members of other life kingdoms: in different yeast species, protozoa, bryophytes, marine brown, red and green algae. Properties of the Na^+-ATPases from different organisms may differ considerably. For instance, Na^+-ATPases from some marine algae (*Heterosigma akashiwo, Porphyra yezoensis,* others) are similar to the mammalian Na^+,K^+-ATPase. Yeast enzymes differ from the mammalian Na^+,K^+-ATPase and form particular group of ENA ATPases.
Our effort has been aimed at the investigation of the sodium pumps in two green microalga species, *Tetraselmis viridis* and *Dunaliella maritima*. These algae inhabit sea water and Na^+-translocating ATPases at the plasma membrane can be envisaged as an evolutionary adaptation to high salinity. The experiments on the Na^+-ATPase functional identification were performed using inverted plasma membrane (PM) vesicles isolated from the algal cells. We demonstrated that the PM vesicles were able to accumulate Na^+ in an ATP-dependent and $\Delta\mu H^+$-independent manner. This finding supported the idea that the primary Na^+-pumps, Na^+-ATPases function in the PM of these organisms. Detailed investigations showed that the ATPases have both similarities and differences. The enzymes are electrogenic pumps belonging to the family of P-type ATPases. They (i) operate in the weakly alkaline pH region with maximal activity at pH 7.5 – 8.0; (ii) are possessed of similar affinities to Na^+ with app. K_m of about 5mM; (iii) are Mg^{2+}-dependent enzymes with high affinity to this ion (K_m of about 60μM). The enzyme from *T. viridis* is the Na^+,H^+-ATPase, that exchanges mNa^+ for nH^+ (m>n) during its catalytic cycle, but the enzyme from *D. maritima* is an electrogenic uniporter for sodium ion. The cDNA of *T. viridis* Na^+-ATPase was cloned (GenBank ID: FN 691482.1). Phylogenetic analysis revealed that *T. viridis* Na^+-ATPase clusters in the phylogenetic group of the fungal Na^+-transporting ENA ATPases. The work on identification of the Na^+-ATPase gene from *D. maritima* is in progress.
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The actin cytoskeleton is a target for NaCl and hydroxyl radicals in *Arabidopsis thaliana* root cells

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The effect of NaCl on root cells is a prime cause of plant damage by salinity. In *Arabidopsis thaliana* root cells, early effects of NaCl (20-400 mM) include the elevation of cytosolic free Ca^{2+}, generation of hydroxyl radicals and K^+ efflux, which together trigger adaptation programmes, metabolic adjustment or a programmed cell death (Demidchik et al., 2014, DOI: 10.1093/jxb/eru004). Using *GFP-FABD2 Arabidopsis* roots and confocal microscopy, we have found that the cytoskeleton is another prime target for NaCl. Here, we have demonstrated that 100 mM NaCl delays or stops root growth and induces actin polymerization in the root elongation zone within 10 min. Microfilament angle distribution deviated

from an initial orientation (predominantly axial) to a broad spectrum with peaks at 15°, 45° and 90°. This effect was prevented by addition of polyamines (spermine and spermidine), blockers of Ca^{2+}-permeable cation channels and scavengers of hydroxyl radicals. Therefore, it was probably related to Ca^{2+} influx and hydroxyl radical generation. Addition of hydroxyl radical-generating mixture, containing 1 mM Cu^{2+}, 1 mM L-ascorbic acid and 1 mM H_2O_2, resulted in qualitatively similar cytoskeleton rearrangements, which however were about ten times more rapid (as compared to NaCl-induced effect). Treatments with hydroxyl radical scavengers, polyamines, EGTA or by cation channel blockers delayed the hydroxyl radical-induced effect on the root cell cytoskeleton. These ameliorating treatments also rescued root growth arrest induced by NaCl or oxidative stress. We propose a hypothetical model that relates the observed cytoskeleton rearrangements with other early stress-induced physiological processes in plant cells.

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The transcription factors and COR/LEA proteins gene expression in wheat under separate and combined effect of low temperature and cadmium

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A range of stress factors affects plants and leads to the induction of a signaling cascade that causes to the activation of gene expression and synthesis of the proteins involved in plant defense reactions. The regulatory proteins - transcription factors (TFs) play an important regulatory role in signal transduction, which in turn are able to induce or inhibit the expression of other genes. It have been shown that the expression of transcription factors (*CBF1, DREB1, MYB80*) genes in the leaves of wheat (*Triticum aestivum* L.) was activated in the initial period (minutes, hours) with low temperature (4°C) and cadmium (100μM) influence as well as under their combined effect, and remains elevated during 7 days. It is noted that the *MYB80* gene mRNA content was higher in plants that were subject to cadmium. This may be due to the fact that some representatives of the MYB TF family, along with the participation in the cold resistance of plants, may be a negative regulator of the expression of TFs *CBF* and *DREB* genes. This may explain a greater activation and expression of *CBF* and *DREB* genes than *MYB* under cold conditions. It is known that the target genes for CBF and DREB TF are genes encoding COR / LEA proteins that play an important role in plant cold tolerance. In the our experiments, the ABA-dependent and ABA-independent signaling pathway were analyzed. In particular, the analysis of the accumulation of mRNA gene *COR/LEA* proteins showed that accumulation of gene transcripts (*WRAB15, WRAB18, WCOR15, WDHN13*) increased in the initial period of the separate and combined effect of low temperature and cadmium. Meanwhile the transcript level of *WCS120* gene accumulation was observed only under the low temperature and its coactions with the cadmium whereas in presence of cadmium the increase in *WCS120* gene expression was not detected. With this in mind, it can be concluded that the adaptation of plants to separate and combined effect of low temperature and cadmium is associated with activation of the gene expression of TFs (*CBF1*, *DREB1*, *MYB80)* and COR/LEA proteins (*WRAB15*, *WRAB18*, *WCOR15*, *WDHN13*). By contrast, the accumulation of *WCS120* gene transcripts was largely related to the increase plant resistance to low temperatures than to cadmium.

Proline-hydroxyproline-rich antimicrobial glycopeptides from dandelion (*Taraxacum officinale* Wigg.) flowers demonstrate a signaling function

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Plants deploy a sophisticated multi-level immune system to combat pathogens and pests. Receptors at the plant cell surface perceive signaling molecules from host and non-host pathogens and from their own damaged cells. Recognition of pathogen- or plant-derived molecules triggers the innate immunity response by up-regulation of defense-related genes. Once recognized by these receptors, the plant immune cascade is initiated. In present study, we describe two novel cysteine-free proline-hydroxyproline-rich glycosylated AMPs named ToHyp1 and ToHyp2 from the dandelion flowers that possess both antimicrobial and signaling activity. We investigated the involvement of ToHyp2 peptide in induction of defense reactions on detached leaves of three susceptible wheat cultivars (Mironovskaya 808, Khakasskaya and Arkas). Leaves were treated with the peptide solution for 24 h, whereupon the peptide was washed from the leaf surface, and the leaves were inoculated with Septoria nodorum spore suspension. Disease development was monitored 5-6 days after inoculation. In Mironovskaya 808, progress of the disease was delayed by 29-33%. A two-fold increase in the peptide concentration did not result in a more pronounced effect. Similar experiments with other wheat cultivars Khakasskaya and Arkas showed that they were less sensitive to the pretreatment with the peptide. The reduction in disease symptoms was 17% and 13%, for Khakasskaya and Arkas, respectively. To test whether ToHyp2 peptide exhibits defense-signaling activity, an alkalinization assay developed to detect bioactive peptides was employed. It has been shown that plant signaling peptides induce alkalization of the medium caused by the blockage of a proton pump in the cell membranes. So we assayed the ability of ToHyp2 to induce a pH shift in 10- and 12-day-old rice suspension cultures. Chitosan was used as a positive control. ToHyp2 induced alkalization of the medium although much less effectively than chitosan. The effect was dependent on the peptide concentration. In 10-day-old culture, the peptide at a concentration of 60 μg/mL caused a pH shift of 0.25. The alkalinization of the medium peaked at about 60-70 min and then slowly declined. An increase in the peptide concentration to 90 μg/mL produced a more clear-cut effect of 0.3 pH units in the 12-day-old culture. The response peaked earlier, at 50 min and then slowly declined. The ToHyp2 peptide displays signaling activity although it is rather weak, possibly due to poor recognition of the peptide by the rice receptor in cell membranes. In support of this suggestion, no alkalinization of the tobacco suspension culture medium was observed in the presence of tomato systemin, providing evidence for the lack of interactions between the tomato peptide and tobacco cell receptors. Inspection of ToHyp2 structure peculiarities showed that it resembles plant signaling peptides. So it was tempting to investigate whether the peptide has a role in defense-related signaling. With this aim in view, we tested the ability of ToHyp2 to induce defense reactions using two in vitro assays. In the first assay, excised wheat leaves were pretreated with the peptide solution, whereupon they were infected with *S. nodorum* spores, and the disease development was monitored. In three susceptible wheat cultivars, the peptide caused a delay in symptom development pointing to the role of the peptide in triggering defense response. These results were complemented by the alkalinization assay used to detect signaling peptides. The results showed that the peptide induced alkalinization of the cell suspension culture medium characteristic of signaling peptides, although at much higher concentrations than a well-known elicitor of defense reactions chitosan. Classical signaling peptides act at nanomolar concentrations.

Isolation and Characterization of Two Drought Responsive Genes from Mulberry

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Abiotic stresses such as salinity, cold and drought are the key factors inhibiting plants from growing to their full potential. The early responsive to dehydration (ERD) genes were isolated on the basis of their ability of being rapidly induced by drought stress. ERD15 and related proteins belong to a small, ubiquitous gene family specific to plants. Since these genes are rapidly induced by drought stress, they

are suggested to play an important role during drought stress. Plant lectins are characterized as proteins that contain a non-catalytic domain that binds reversibly with a mono- or oligosaccharide. Recent trends have suggested a possible connection between lectin genes and abiotic/biotic stress responses. The expression of MiERD15 and Mannose Binding Lectin (MiMBL*)* is induced by abiotic stress conditions. MiERD15 showed transactivation potential in Y1H assay suggesting that it can function as a stress induced transcription factor. Thus, in order to understand the putative functions of these genes in mulberry, we isolated and characterized MiERD15 and MiMBL and their upstream regulatory regions (URR). Our analysis collectively suggests that these URRs can act as efficient promoters by inducing expression under normal conditions which is retained at higher levels under osmotic stress inducing treatments. The present study, thus, identifies these novel genes and furthers our understanding of their function especially under abiotic stress conditions.

Membrane transporters mediating root signalling and adaptive responses to oxygen deprivation and soil flooding

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The lack of oxygen in flooded soils blocks aerobic respiration and ATP synthesis in mitochondria, with major implications to root metabolism and nutrient acquisition. Excess water also causes a sharp decrease in soil redox potential, causing elemental and metabolite toxicities. Finally, hypoxic conditions favour generation of reactive oxygen species (ROS). These ROS can each damage to plant cells and tissues by causing lipid peroxidation in membranes, DNA damage, protein denaturation, carbohydrate oxidation, pigment breakdown and an impairment of enzymatic activity. In this work, we discuss the ionic and molecular mechanisms underlying plant adaptive responses to above factors and elucidate the role of the plasma- and organelle-based membrane transporters in plant adaptive responses to flooding. We show that energy availability and metabolic shifts under hypoxia and anoxia are critical in regulating membrane-transport activity. We illustrate the high tissue- and time-dependence of this regulation, reveal the molecular identity of transporters involved and discuss the modes of their regulation. We show that both reduced oxygen availability and accumulation of transition metals in flooded roots result in a reduction in the cytosolic K^+ pool, ultimately determining the cell's fate and transition to PCD. The latter process may be of adaptive significance, contributing to aerenchyma formation by a mechanism unrelated to lysogeny. This process can be strongly affected by hypoxia-induced changes in the amino acid pool profile and, specifically, GABA accumulation. It is suggested that GABA plays an important regulatory role, allowing plants to proceed with H_2O_2 signaling to activate a cascade of genes that mediate plant adaptation to flooding while at the same time, preventing the cell from entering a "suicide program". We further link these changes with the tissue-specific root ion profiling, to reveal essential roles of the CAX and ACA calcium transport systems for hypoxia response in plants.

Ca^{2+} and H^+ signaling in early auxin transduction.

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Rapid shift in cytosolic Ca^{2+} and H^+ concentration is wildly known specific auxin induced reaction. We estimated a time-priority of Ca^{2+} changes for cells, sensitive to auxin and able to elongate. It is due to the activation of Ca^{2+} transport through plasma membrane. According to the experiments with *tir1* and *axr1-3 Arabidopsis* mutants, the amplitude of Ca^{2+} elevation did not depend on auxin perception which is employed TIR1 receptor. On the opposite, the modulation of ABP1 concentration (external application, antisense transformation, *etc*.) resulted in the modulation of auxin-induced Ca^{2+} rise in

cytosol. This data is especially important because of re-evaluation of physiological role of ABP1, which is for several decades was supposed to be a part of plasma membrane auxin receptor.
Further auxin-triggered cytosol acidification is greatly depends on the activity of plasma membrane H^+-ATPase. Analysis of possible changes in enzyme facilities revealed non-linear alteration in hydrolytic and transport activity during the process of elongation growth. Changes in the activity correlate with H^+-ATPase regulation at transcription and post-translation level.
It can be concluded that TIR1-related hormone perception and transduction pathway does not include Ca^{2+} and H^+ signaling. While ABP1, shown to be important in cell elongation. According to our data early transduction events strongly required Ca^{2+} ion transport through plasma membrane and implication of plasma membrane H^+-ATPase for acidification. This coincides with early suggested model of plasmalemma receptor, with ABP1 as an associative domain.

Cold-induced changes in raft-forming lipids and activity of tasmt1 gene in wheat seedlings

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The plasma membrane of higher plants contains a complex mixture of sterols. The predominant phytosterols are *β*-sitosterol, campesterol, stigmasterol, and the presence of cholesterol has also been demonstrated. Due to the high affinity of sterols and sphingolipids, these two lipid classes can form special high density platforms, or "lipid rafts", and therefore are often called as raft-forming lipids. Lipid rafts are known to be involved in cell signaling. Recently, we demonstrated that the reduction of sterol content in *Triticum aestivum* roots caused by sterol-binding agents methyl-*β*-cyclodextrine (M*β*CD) and nystatin was accompanied by a significant increase in the total content of glycoceramides (GlCer). In this work, we analyzed the effects of cold stress on the composition of raft-forming lipids and the activity of *SMT1*, a gene of sterol biosynthesis in wheat seedlings. The level of sterols and the pattern of molecular species in roots and leaves were time-dependent. Short-term cold response (1 h, +4°C) was characterized by a significant increase in total sterols and particularly ethyl sterols. These changes were more prominent in leaves than in roots. Long-term response (12 h, +4°C) was characterized by a reduction of the level of sterols to initial values both in roots and leaves. Notably, the changes in sterol levels were accompanied with opposite alterations in the content of GlCer with a drop after 1 h and a rise after 12 h of cold treatment.
Cold also affected the activity of a gene encoding C24-sterol methyltransferase (SMT), a key enzyme of sterol biosynthesis. We found that *SMT1* gene in the allohexaploid wheat genome (AABBDD) is presented in three homoeologous copies, which were differentially expressed in roots and leaves in response to cold. Real time PCR analysis demonstrated up-regulation of *TaSMT1*-4D, while *TaSMT1*-5A was constitutively expressed and did not show cold sensitivity, and *TaSMT1*-4B was not activated. Sequencing and analysis of a promoter region showed that activation of *TaSMT1*-4D in the response to cold is regulated by LTR, the *cis*-element involved in low-temperature responsiveness and other stress-responsive motifs.
In conclusion, the interrelated changes in sterols and GlCer in response to cold stress may suggest their involvement in cold signaling in plants.
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Mechanisms of light stress tolerance in barley mutant plants lacking chlorophyll b

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Chlorophyll *b* is emerging as the major regulator of the size and stability of antennae complexes in land plants (Tanaka&Tanaka 2011 Biochim Biophys Acta). The inability to synthesize chlorophyll *b* leads to defects in composition and functions of antennae and thereby to impaired light harvesting and photoprotection. Consequently, chlorophyll *b*-less mutants usually display low levels of photosynthesis, poor growth and yield. They also exhibit an increased light sensitivity and are more prone to photoinhibition than the wild type (Leverenz et al. 1992 Physiol Plant; Lin et al. 2009 Photosynthetica; Dall'Osto et al. 2010 Mol Plant). In some of these mutants, ROS levels in illuminated leaves were studied and increased levels of singlet oxygen production found (Ramel et al. 2013 Plant Cell).
We studied the barley mutant *chlorina f2*^{3613} lacking functional chlorophyllide-*a*-oxygenase (Mueller et al. 2012 Plant Cell Physiol) grown in the field. This mutant showed all typical characteristics of chlorophyll *b*-less mutants, such as low photosynthesis, growth and yield, and displayed high levels of singlet oxygen production in leaves in the light. However, when the excitation pressure experienced by *chlorina f2*^{3613} plants was temporarily decreased by shading the field-grown plants for one week, a doubling of chlorophyll *a* content and an increase in the CO_2 fixation rate were observed. The high levels of photosynthesis and pigments were maintained until the end of the growth period, resulting in an increase of vegetative mass production and a seed yield comparable to that of the wild type (Tyutereva&Voitsekhovskaja 2011 Russ J Plant Physiol). Accordingly, the levels of singlet oxygen production in the light decreased in the leaves of these plants after shading.
We searched for the mechanisms allowing *chlorina f2*^{3613} to develop the light stress tolerance similar to that of the wild type. We analyzed protein composition of the antennae, ultrastructure of chloroplasts, and the NPQ levels. Transcript patterns were compared in both phenotypes (light-sensitive and light-tolerant) of *chlorina f2* ^{613} *vs.* the wild type. The rates of diffusion of pigment-protein complexes and of lipids in the thylakoid membranes were studied by FRAP. The data suggest a link between the redox state of the PQ pool, the synthesis of several minor antenna proteins, the changed size and composition of the PSII-based supercomplexes in the grana membranes and the improved diffusion in the thylakoid membranes in the light-tolerant phenotype of the barley mutant *chlorina f2*^{3613}.
Financial support by the Russian Science Foundation (grant #14-16-00120 to OVV) is gratefully acknowledged. We used equipment of the Core Facility Center "Cell and Molecular Technologies in Plant Science" at the Komarov Botanical Institute RAS and the research resource center "Molecular and cell technologies" of St. Petersburg State University.

Moss *Physcomitrella patens* as a model system for plant molecular biology

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When compared to other model plants, the moss *Physcomitrella patens* (*Physcomitrella*) has several unique features including high frequency of homologous recombination, haploid state of gametophyte and rapid growth of protonema filaments, which makes it an excellent plant model for genetic studies, as well as biotechnological applications. Throught homologous recombination can be easily prepared knockout mutants / targeted knockout of genes. Predominant haploid state during life cycle allows strightforward identification of phenotype changes due to genome alteration. *Physcomitrella* is due to its simple structure suitable for the study of growth and evolution. Early stages of protonema short fragment filamentous tissue has high amount of dividing terminal cells, which are in higher plants limited fraction of dividing cells of apical meristemes.
Physcomitrella has been used as an experimental organism for more than 80 years. Within the last fifteen years, the interest in *Physcomitrella* has rised significantly because of its unique features - simple and easy handling, several options for long-term storage and knowledge of the genome sequence, which is free available in the internet database Cosmoss.

Defending plants against the World's most pesticide-resistant insect, *Myzus persicae*: A role for calcium

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Myzus persicae (the green peach aphid) holds the Guinness World Record as the most resistant insect to pesticides, documented as being resistant to 71 different varieties, and acts as a vector for over 100 plant viruses. Upon perception of *M. persicae,* plants activate pattern-triggered immunity, a pivotal part of which is hypothesised to be calcium signalling. However, the mechanism by which this works is still largely unknown. Using the latest *in vivo* imaging techniques, we have been able to visualise in real time a plant calcium burst around the site of aphid feeding, with high spatial and temporal resolution. This burst is extremely rapid, placing calcium release as one of the first events in the plant-aphid interaction. We have also shown that it is reliant on vacuolar calcium stores, with loss of TWO PORE CHANNEL 1 (TPC1) significantly reducing the calcium burst, and over-activation of the channel greatly enhancing it. Furthermore, we have linked the calcium release to specific feeding phases of *M. persicae* and have evidence that feeding is significantly altered on mutants of TPC1. Our current work centres on identifying additional channels that contribute to this response and identifying the downstream targets of the signal. Our results suggest that calcium signalling via TPC1 significantly contributes to aphid performance on plants, and may hold the key to understanding the role of such signalling in the plant defence response.

Sensing and signalling salt stress in plants

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Soil salinity is a major environmental constraint to crop production that cost agriculture estimated US$ 27.3 billion p.a. in lost opportunities. While the molecular identity and a functional expression of Na^+ transport system mediating Na^+ exclusion from the cytosol was studied in details over the last decade, much less is known about mechanisms by which plants sense high Na^+ levels in the rhizosphere. In this work, we summarize our current knowledge for the molecular identity of the possible candidates for this role. The list includes: Na^+ transport systems; mechanosensory proteins; proteins with regulatory Na^+ binding sites; and sensing mediated by cyclic nucleoties, annexins, purines and voltage gating. It is suggested that in most cases several transport proteins are clustered together to form a "microdomain" in a lipid raft, allowing a rapid change in activity of one of them be translated into stress-induced Ca^{2+} and H_2O_2 'signatures". We then discuss pathways of stress signalling to downstream targets and compare kinetics and specificity of salt stress signalling in various cell types. Using bread wheat genotypes contrasting in salinity stress tolerance, we used CoroNa Green fluorescent confocal dye imaging method to investigate the essentiality of the vacuolar Na^+ sequestration between functionally different root tissues, and link it with the overall salinity stress tolerance in this species. Our major observations were as follows: 1) salinity stress tolerance correlated positively with vacuolar Na^+ sequestration ability in the mature root zone but not in the root apex; 2) Contrary to expectations, cytosolic Na^+ levels in root meristem were significantly higher in salt tolerant than sensitive group, while vacuolar Na^+ levels showed an opposite trend. These results are interpreted as meristem cells playing a role of the "salt sensor"; 3) No significant difference in the vacuolar Na^+ sequestration ability was found between sensitive and tolerant group in either transition or elongation zones; 4) The overall Na^+ accumulation was highest in the elongation zone, suggesting its role in osmotic adjustment and turgor maintenance required to drive root expansion growth. Taken together, the reported results suggest high tissue-specificity of Na^+ uptake, signalling, and sequestration in plant roots. The implications of these findings for plant breeding for salinity stress tolerance are discussed.

The role of polyamines in signaling and adaptation to oxygen deprivation and subsequent re-aeration in plants

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The aliphatic diamines (putrescine, cadaverine, diaminopropane) and polyamines (spermidine and spermine) are found in all living creatures. Diamines and polyamines usually fulfill similar physiological functions and are considered as one group of compounds - polyamines (PAs). The most abundant PAs in plants are Put, Spd and Spm. PAs are involved in various processes associated with plant growth and development along with stress signaling and protection. We studied the levels of endogenous PAs in plant tissues and the effects of exogenous PA treatment on plant viability and lipid peroxidation under anoxia and post-anoxic oxidative stress in wheat and rice seedlings.
The initial PA levels were similar in tissues of wheat and rice. Imposition of anoxia and subsequent re-aeration led to considerable accumulation of diamines (Put, Cad and DAP) in wheat seedlings, whereas of polyamines (particularly Spm) in rice. DAP appeared in plants only during stress response; and its level corresponded stress intensity and was higher in less tolerant plant (wheat). PAs interact with different signaling cascades by affecting concentrations of reactive oxygen species, ethylene, auxin and abscisic acid. Different pattern of PA accumulation in wheat and rice could trigger various signaling cascades resulting in dissimilar resistance and adaptation to oxygen deficiency.
To check the protective action of PAs on plant tissues after oxygen deprivation we used two methods of plant viability assessment: electrolyte leakage test and tetrazolium test. Both tests showed similar results that all studied PAs improved survivability of wheat seedlings and had no significant effect on rice ones. Moreover, investigation of lipid peroxidation also revealed positive action of PA treatment mainly in wheat. Put, Cad and Spm were the most effective.

NaCl-induced generation of superoxide and DNA breaks in *Physcomitrella patens*

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Only 2% of plants can survive under salinity conditions. One prominent example is a moss *Physcomitrella patens*, which is a great model organism for plant physiology and evolution studies. Green algae that are ancestors of mosses lived in saline water and had an efficient protection against NaCl. Higher plants lost their protection against NaCl while some mosses still have them. Mosses were first terrestrial plants and share many physiological features with salt-tolerant alga. In this respect, study of salt response in mosses can reveal hidden fundamental mechanisms of salt tolerance.
Here, we have investigated in details a key primary reaction of *Physcomitrella patens* (*P. patens*) to NaCl, the generation of superoxide and measured the genotoxic effect of NaCl using Comet Assay. To examine superoxide production, the fluorescent probe dihydroethidium (DHE) was used. *P. patens* tolerate as much as 200-500 mM NaCl therefore relatively high NaCl concentrations were used in this study. We have found that NaCl at concentrations above 200 mM caused significant increase in an intensity of DHE fluorescence (up to 150% as compared to background values). The effect of NaCl increased with NaCl concentration, reaching the maximal value at 300 mM. Accordingly to manufacturer's guidance DHE is selective to $O_2^{\bullet-}$, however our tests demonstrated that this probe is sensitive to a number of ROS. The superoxide dismutase decreased NaCl-induced DHE fluorescence by 40-45% and 60% at 200-300 mM NaCl and 400 mM NaCl, respectively. These data show that at least a half of DHE signal originated from superoxide, while another half was caused by other ROS. Thiourea, which is a specific $^{\bullet}OH$-scavenging agent, reduced NaCl-induced DHE fluorescence by 20% at 200 mM NaCl and by 30% at 300 and 400 mM NaCl, respectively. This indicates that significant portion of DHE signal was due to reacting with hydroxyl radicals. Reduced glutathione, dimethyl sulfoxide and spermine modified NaCl-induced DHE signal. They caused 40-50% reduction in DHE signal at 200-300 mM NaCl and 25-30% at 400 mM NaCl, respectively.
Treatments of plants by 300 and 500 mM NaCl (3 h) triggered increase in double strand DNA breaks (based on COMET assay) from 11.5% to 19.6%. Single strand DNA breaks also doubled as compared to control. 0.1-1% DMSO reduced the effect of NaCl on double and single strand DNA breaks. This

strongly suggests that NaCl-induced ROS are capable of damaging DNA through both double and single strand breaks.
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SESSION 5

Programmed Cell Death and Autophagy

The role of programmed cell death in the secretion of growth regulating signal substances in wheat embryogenic calli

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The key moment in the investigation of somatic embryogenesis process is study of its very early stages. It is crucial to clarify not only the signals and inductors of this process, but also the mechanisms that force differentiated cell *in vitro* to switch over to other developemental pathway (Butenko, 1999). Because the long-term embyogenic calli have a single cell origin of embyoids and appeared to be a very responsive to morphogenesis regulations by phytohormones and trophic factors, they have become a very useful model system for investigation of these issues.

Effect of phytohormones on the composition of cell populations of wheat and barley long-term FE calli, compared to friable and compact non-embyogenic tissues, have been investigated in this work by the methods of light and electrone microscopy. As a result, we found the distinct features of embyogenic tissues in comparison with non-embyogenic calli: the presence of cells with signs of programmed cell death (PCD) or apoptosis, accumulation of dense net polysaccharides in the extracellular space, separation of spherical embyogenic competent cells covered by callose envelope. It has been shown, that the increase of the proportion of cells with signs of PCD in FE tissues under the effect of 2,4-D is accompanied by enhanced accumulation of extracellular polysaccharides (EPS) as well as by the stimulation of calli's growth and embyogenic potential. It has been determined by light and electrone microscopy that cells with signs of PCD secrete into the extracellular space EPS with proteoglycan nature during the course of their death. Bioassays *in vitro* and *in vivo* revealed that fractions of the secreted EPS possess antiauxin activity, i.e. they inhibit the cells'elongation, stimulated by 2,4-D; cause separation of callus cells by means of callose coat and their reprogramming into the embyoidogenic developmental pathway; stimulate the growth of callus tissues, increase the stress tolerance of plant seeds.

On the basis of fulfilled research we suggest hypothetical scheme, that demonstrates the cyclic character of the initiation and desintegration processes of somatic embyoids in the long-term totipotent embyogenic calli. According to this scheme, the sequence of events, caused by 2,4-D, as the following. High concentrations of 2,4-D (5,0-7,0 mg/l) effect on callus cells as strong stress factors, blocking differentiation processes of four-, eight- celled proembryos and globules, causing their desintegration. During this process embryoids are destructed onto the cells with signs of PCD and single competent cells, which under the effect of EPS enter the path of embryoidogenesis again. Initiated proembryos also a capable to dissociate through the PCD process with the formation of spherical competent cells. By this way embyogenic potential of calli is constantly maintained during multiple subcultivations. The decrease of 2,4-D concentration up to 1,0 mg/l diminishes stressful influence of phytohormone, and cell death does not occur. Somatic embyoids are able in this case to normal developement and differentiation with the formation of the whole plant.

Overall, we have discovered the important role of PCD and polysaccharides secretion accompanying this process in the regulation of cells' shape and size, in the switching over callus cells to the embyoidogenic developmenthal pathway, in the maintenance of embyogenic competent cells' pool, regulation of calli' growth and stress resistance. It is important to point out that biological activity of fractions in conditions of salt stress was shown in the nano- and picomolar concentrations, and, in some cases, had saltatory character. Both of these features are characteristic of signaling molecules. Therefore, we assume that PCD and polysaccharides' secretion could be a parts of signaling cascade in the process of hormone regulated morphogenesis in vitro.

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Polyamines induce programmed cell death in *Arabidopsis thaliana* roots

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Introduction. Polyamines are water-soluble aliphatic compounds containing several amine groups and having fundamental importance in plant stress biology. Putrescine (diamine), spermidine (triamine) and spermine (tetramine) are the most widespread and physiological important polyamines in plants. The level of these polyamines in tissues increase in response to almost any abiotic stress. Some studies showed that the symptoms of oxidative stress decrease in plants overexpressing enzymes of polyamine biosynthesis. However polyamines also act as substrates for biosynthesis of ROS in apoplast by polyamine oxidase. Perhaps, pro- or antioxidant effects of polyamines depend on exact physiological state and environmental conditions. It is difficult to predict how exogenous polyamines can modify cell viability and whether they can induce the programmed cell death (PCD). PCD is a crucial reaction in plant stress responses and its regulation by polyamines may have fundamental significance.
The aim of this study was to determine symptoms of PCD in *Arabidopsis thaliana* root cells in the presence of exogenous spermine, spermidine and putrescine.
Materials and methods. Roots of *Arabidopsis thaliana* L. *Heynh* ecotype WS-0 (Wassilewskija) were used. They were grown vertically on Petri dishes using standard sterile conditions. PCD symptoms were assessed in control and after treatment with 0,01-1 mM spermine, spermidine and putrescine. The effect of polyamines on PCD was also examined in the presence of 0.3% dimethyl sulfoxide (DMSO), 1 mM thiourea, 600 units ml^{-1} superoxide dismutase (SOD) and 1000 units ml^{-1} catalase. Morphological symptoms of PCD were identified and compared in atrichoblasts and trichoblasts. Generation of ROS (superoxide) was measured using a fluorescent probe dihydroethidium (10^{-6} M; Sigma, USA).
Results. All polyamines significantly inhibited elongation of Arabidopsis roots at 0,03-0,3 mM. 1 mM spemine and spermidine stopped root growth. The amount of root cells with PCD symptoms (protoplasm shrinkage, plasma membrane damages, dark areas, etc.) in control did not exceed 10%. Quantity of trichoblasts with PCD symptoms increased significantly when seedlings were exposed to 0,03 mM spermine, 0,1 mM spermidine, and 0,1 mM putrescine. Similar effect was observed in atrichoblasts. The addition of ROS scavengers inhibited the development of PCD caused by 0,3 mM putrescine, but did not change the influence of spermine. The effect of spermidine was sensitive to SOD and DMSO. Tests with dihydroethidium showed that 0,3 mM spermine stimulated superoxide generation in root cells while spermidine did not change superoxide productions. Intriguingly, putrescine significantly inhibited superoxide generation.
Conclusions. Treatment with exogenous polyamines triggers PCD. Polyamine-induced PCD is inhibited by antioxidants, such as SOD and DMSO. Polyamines have contrasting effects on superoxide production: spermine stimulates it, spermidine does not affect this process while causes its inhibition.
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Phytaspases: role in plant cell death and beyond

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Phytaspases are subtilisin-like plant proteases exhibiting peculiar aspartate ('caspase-like') substrate specificity. Being synthesized as inactive precursor proteins, phytaspase proenzymes are constitutively and autocatalytically processed to generate mature enzymes which are secreted into the apoplast [1]. Induction of programmed cell death (PCD), however, triggers phytaspase re-localization into plant cells. In accord with this behavior, phytaspases are involved in the accomplishment of plant PCD induced by biotic and abiotic stresses [1,2]. We will discuss this plant strategy to control PCD, which is distinct from that of animals. Emerging evidence for phytaspase-mediated fragmentation of protein targets and possible consequences of these cleavage reactions will also be presented.
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RNA turnover in the plant vacuole via autophagy

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The degradation of cellular components by autophagy is critical for plant cells to remove damaged components and maintain functional intracellular contents. Ribosomes represent a significant metabolic investment, as they contain a majority of a cell's RNA and large quantities of its protein; however, little is known about their turnover in plants.
RNase T2 enzymes represent a widely conserved class of ribonucleases present in the genomes of nearly all eukaryotes tested. We show that an Arabidopsis T2 RNase, RNS2, and autophagy function together in the degradation of RNA within the vacuole in normal growth conditions. Disruption of RNS2 leads to increased basal autophagy, with production of autophagosomes containing RNA and ribosomes. *rns2* mutants accumulate ribosomal RNA within the vacuole, and this accumulation is eliminated in an *atg5* autophagy mutant background, indicating that autophagy is used for rRNA transport into the vacuole for degradation. Analysis of an enzymatically active but mislocalized RNS2 mutant demonstrated that vacuolar localization is required for RNS2 function. A combination of transcriptome and metabolome analysis of an *rns2* mutant suggested that the pentose phosphate pathway is activated. These data support a model in which either ribosomal RNA or ribosomal subunits are transported into the vacuole by an autophagy-related process requiring ATG5, followed by degradation and recycling via a pathway involving RNS2. We propose that lack of rRNA recycling in *rns2* cells triggers a change in carbon flux, which is redirected through the PPP to produce ribose-5-P for de novo nucleoside synthesis. rRNA or ribosome turnover is thus essential to maintain nucleoside homeostasis.

Molecular composition of stress granules in Arabidopsis

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Efficient adaptation to stress depends on the availability of energy resources. Stress drives cells to an energy crisis whereupon they have to reduce energy expenditure in order to survive. To this end, eukaryotic cells compartmentalize specific mRNAs and proteins in cytoplasmic ribonucleoprotein complexes known as stress granules (SGs)[1]. In these structures mRNA molecules are stored, degraded or kept silent in order to prevent energy expenditure on producing useless, surplus or even harmful proteins under stress conditions[2]. Molecular composition, structure, and function of SGs in plants are largely unknown. Recently, we have revealed that Tudor staphylococcal nuclease (TSN) is essential for the integrity and function of SGs in *Arabidopsis thaliana*[3]. Yet, TSN is stably associated with SGs, suggesting that it may serve scaffolding role to recruit other proteins to the mRNP complexes. Therefore we used TSN as bait in tandem affinity purification of SGs-associated proteins. Localization of identified proteins to SGs *in vivo* has been further verified by live imaging techniques. We have finally obtained a list of SGs-associated proteins.

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Measurements of NaCl-, heavy metal- and hydroxyl radical-induced programmed cell death in *Arabidopsis thaliana* roots

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Introduction. Programmed cell death (PCD) is a crucial process in all eukaryotes which plays an important role in developmental pathways and survival of organisms. PCD is also a defense mechanism against stresses. However, it is not always beneficial to plants and can lead to severe loss in crop yield. Understanding of PCD mechanisms is essential not only for development of fundamental concepts on ontogenetic processes in plants, but also for creating means of control and stimulation of plant productivity and stress resistance.
The aim of this study was to determine the role of systems located in the plasma membrane of plant root cells in the development of stress-induced PCD.
Materials and Methods. The following lines of *Arabidopsis thaliana* L. were used: WS-0 (Wild Type: WT), *gork1-1* (lacking root K^+ efflux channel GORK) and *rhd2* (lacking root ROS-producing enzyme NADPH oxidase C). Morphological symptoms of PCD were studied in root atrichoblasts and trichoblasts (root hairs). Viability tests were conducted using Evans Blue (Sigma, USA), Fluorescein diacetate (Sigma, USA) and Nikon epifluorescent microscopy. Cell death protease activity was measured using CaspACE™ FITC-VAD-FMK *In Situ* Marker (Promega, USA).
Results. We have designed a set of robust cell morphology tests to examine stress-induced PCD. This was based on detection of protoplasm condensation, appearance of condensed dark areas in place of nuclei, plasma membrane damage, etc.. We have also adapted very sensitive test of caspase-like protease activity (FITC-VAD-fmk). These two tests showed a great sensitivity in early detection of PCD symptoms induced by oxidative stress (Cu^{2+}/L-ascorbic acid), Ni^{2+} or NaCl. The viability tests with Evans Blue and fluorescein diacetate probes displayed high sensitivity for detection of PCD induced by oxidative stress and NaCl, but these probes did not report cell death caused by Ni^{2+}. Designed techniques allowed high throughout detection of cells with PCD symptoms in different plant lines. Using these techniques, we have found that *gork1-1* and GORK with modified ROS sensing center as well as *rhd2* have delayed PCD development in response to a number of stresses. This also allowed to examine effects of pharmacological agents, such as thiourea, Gd^+, tetraethylammonium and others.
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Redox regulation of Autophagy in plants

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Autophagy is a strictly regulated catabolic process that targets damaged or toxic components for vacuolar or lysosomal degradation. In plants, autophagy is involved in development, the response to stress, and programmed cell death. Here we review the intricate link between autophagy and reactive oxygen species (ROS). First, oxidative stress following ROS accumulation is a typical inducer of autophagy. Second, sites of ROS production and signaling are among the primary targets of autophagy. Third, intracellular redox changes can control the formation of autophagosomes by regulating the activity of autophagic (ATG) proteins. Plants use autophagy to survive oxidative stress by degrading and recycling the oxidized proteins and damaged intracellular components, including organelles. For example, our work has shown that in *Triticum aestivim* seedlings oxidative stress induced by the exogenous application of prooxidants such as paraquat and salicylic acid results in macroautophagy. Electron microscopy analysis shows that elimination of mitochondria in autophagolysosomes occurs in paraquat treated wheat roots. Disruption of the mitochondrial electron transport chain by antimycin A, an inhibitor of complex III, causes accumulation of ROS and induces autophagy. The formation of autophagosomes is controlled by the activity of numerous ATG proteins. ATG8, a multifunctional protein from the ubiquitin superfamily, is used as a molecular marker of macroautophagy. The structure of *Ta*ATG8g was found to contain W- and L-sites, necessary for the interactions of ATG8 with various ligands, including ATG4. ATG4 is a cysteine protease and considered to be a direct target for oxidation by H_2O_2. *Ta*ATG4 interacts with *Ta*ATG8 *via* the so-called AIM-motif (ATG8-interacting motif). Modification of ATG8 by the ATG4-mediated cleavage of the C-terminus, a prerequisite for the

formation of autophagosomal membranes, depends on the cellular redox status. Real time PCR analysis demonstrated up-regulation of *TaATG4* and *TaATG8* genes following oxidative stress, wounding, and the disruption of plasma membrane integrity. Redox regulation of the activity of key ATG proteins suggests the existence of a signal transduction pathway where ROS provide a fine tuning control of autophagy during stress response.

The influence of high temperatures of different intensity on gene expression is responsible for programmed cell death development in wheat plants

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Programmed cell death (PCD) is a complicated process and many stress-factors induce it. A lot of transcription factors, enzymes and pro-apoptotic proteins are involved in PCD. This process is underexplored in plants unlike in animals. Thus, we investigated the process of PCD features development and changing of expression of genes involved in PCD (pro-apoptotic gene *Bax* analog, gene of plant metacaspase *McaII* and regulatory gene *RCD1*) in leaves of wheat seedlings, that were subjected to temperatures of different intensity (37 and 43°C).
It was established, that influence of the above-noted temperatures induces DNA fragmentation in leaves of wheat seedling after one day. It points at PCD development. Furthermore, in initial period (15 min) of impact of the temperatures, the accumulation of *Bax* gene transcripts was detected, and then the level of its expression decreased. The content of mRNA of *McaII* increased after one day of 37°C influence and then remained unchanged. The effect of 43°C enhanced the content of mRNA of *McaII* after 15 min of experiment and decreased it after 6 h. It is also important, that the influence of 37 and 43°C resulted in an increased *RCD1* gene expression and this gene is capable of activating other proteins that participate in PCD process. It should be noted, the level of genes expression at the influence of 43° temperature was higher than at the influence of 37°C.
Thus, our data demonstrate that the influence of high temperatures (37 and 43°C) is able to activate the cascade of PCD reactions in leaves of wheat seedling. Probably, the pro-apoptotic gene *Bax* analog appears and functions in an initial stage of this process, *McaII* gets involved in PCD process in later stage. Moreover, the increase of *RCD1* expression is capable of activating other participants of PCD in a late stage of it. The activation of PCD mechanism may be leads to adaptation and maintaining viability of wheat seedlings towards sub-damaging temperature (37°C), but in the same time it may serve as one of the reasons of plants` death at the influence of damaging temperature (43°C).

Age-associated alterations in DNA methylation levels and expression of methyltransferase and demethylase genes in *Arabidopsis thaliana*

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Cytosine DNA methylation is an epigenetic modification that is important for maintaining genome stability and regulating gene expression in higher plants and other organisms. Growing evidence suggest that DNA methylation is implicated in regulating gene expression across plant development and in response to environmental stress. Little is known, however, about the contributions of DNA methylation/demethylation to plant aging and senescence.
The level and pattern of cytosine methylation are determined by both DNA methylation and demethylation machineries. The process of methylation is performed by methylases. Plants possess three methylases families: the METHYLTRANSFERASES (MET), CHROMOMETHYLTRANSFERASES (CMT), and DOMAINS REARRANGED METHYLTRANSFERASES (DRM).
In contrast to DNA methylation, DNA demethylation can be passive and/or active. Whereas passive DNA demethylation may take place due to lack of maintenance of methylation during DNA replication, active demethylation occurs enzymaticaly by removing methylated cytosines. In plants DNA

glycosylases were shown to exhibit the DNA demethylation activity in combination with base excision repair process. Members of the DNA glycosylase family DEMETER (DME) and REPRESSOR OF SILENCING 1 (ROS1) were better studied than DEMETER-LIKE 2 and 3 (DML2 and DML3). Biochemical studies showed that AtROS1, AtDME, AtDML2, and AtDML3 are 5-methylcytosine DNA glycosylases that initiate a base excision pathway for active DNA demethylation in *A. thaliana*.
The purpose of the present study was to elucidate whether nuclear cytosine methylation levels and transcription levels of methyltransferase/demethylase genes change during the life cycle of *A. thaliana*. We used *A. thaliana* to study how increasing chronological age of an annual plant species influences the DNA methylation level. Methylation-sensitive BstHH I restriction digestion was used to investigate DNA methylation level in *A. thaliana* during plant growth, development, and aging. Total DNA was isolated from *A. thaliana* plants 1, 4, 8, and 12 weeks after seed sowing and was digested with BstHH1. Based on methylation-sensitive DNA fragmentation assay, it could be concluded that the chronological aging of *A. thaliana* was accompanied by DNA demethylation.
Using bisulfite sequencing, we analyzed the total level of cytosine methylation within two control nuclear DNA regions (*Actin2* 3'UTR and *ITS1-5.8SrRNA-ITS2* (*ITS*)) of *A. thaliana* plants grown for 1, 4, 8, and 12 weeks. The analysis revealed that the total level of cytosine methylation within the *Actin2* and *ITS* DNA regions gradually decreased with *A. thaliana* growth, maturation, and aging. Bisulfite sequencing revealed that the level of cytosine methylation within the DNA *Actin2* and *ITS* regions decreased with *A. thaliana* growth and ageing. We showed that transcription levels of the methyltransferase genes *AtCMT3* and *AtMETI* significantly decreased during development and ageing of *A. thaliana* plants, while expression of the demethylase genes *AtROS1*, *AtDME*, *AtDML2*, and *AtDML3* increased at least at some stages of plant development.
The data obtained in the present study suggest that the plant DNA regions may also undergo hypomethylation during aging which is likely to affect transcription and genome stability. Plants lack a reserved germ line, and their reproductive structures and gametes form late in growth cycles by differentiation of somatic meristematic cells. Therefore, it is possible that plant somatic cells might pass the accumulated alterations in the DNA methylation patterns to further plant generations, which potentially may influence plant ecological and evolutionary fitness. Further investigations of the occurrence and possible roles of DNA methylation/demethylation processes in plant ageing and senescence are necessary.

Identification and activity of the *ATG8* gene family in wheat

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Autophagic protein ATG8, a molecular marker of autophagy, is involved in the formation of the autophagosomal membrane by a ubiquitination-like process. In contrast to yeast, which only contains one *ATG8* gene, plants have numerous members of the *ATG8* family. The reasons for the presence of such a large *ATG8* family in plants are not entirely clear. Here we searched *in silico* for the wheat *ATG8* genes and analyzed their activity under abiotic stresses, such as oxidative stress, desiccation and wounding. Using URGI BLAST and Phytozome databases, we identified at least nine members of *ATG8* family in hexaploid wheat (*Triticum aestivim* L.). This family was divided into three subfamilies (*ATG8 I, ATG8 II, ATG8 III*). Each subfamily of *ATG8* genes comprises three homoeologous genes, which have similar structures and are located on homoeologous chromosomes (2AS, 2BS and 2DS). The ORF of *ATG8s* consists of five exons and four introns. In the intronic regions we found potential sites of alternative polyadenylation that are involved in the regulation of gene splicing and expression. Real-time PCR analysis showed that all subfamilies of *ATG8* genes are ubiquitously expressed in wheat tissues, with transcripts being several times more abundant in leaves than in roots. It is known that ATG8 proteins are involved in the responses of plants to stresses. Oxidative stress induced in wheat seedlings by the application of the ROS generating agent methyl viologen (MV) caused differential expression of *ATG8* genes. In roots, up-regulation of the *ATG8 I* and *ATG8 III* subfamilies was observed after 6 h and 24 h of MV application respectively, while the activity of *ATG8 II* did not change. In leaves, which are more sensitive to MV treatment, the expression level of all three subfamily *ATG8* genes (especially of *ATG8 I*) was much higher than in roots. Drought stress was accompanied by an inhibition of growth, a decrease in total water content, the accumulation of ROS, and a rise in *ATG8* gene expression. The levels of expression of *ATG8 I* and *ATG8 II* genes were significantly increased by drought stress after 10 d of withholding water. Strong up-regulation of the *ATG8 III* gene subfamily in leaves was observed after 12 d of drought. Wounding stress induced by excision of roots from seedlings stimulated *ATG8* expression

only 24 h after excision, although the formation of autophagosome was visualized after 2 h. Thus, our data suggest that the up-regulation of *ATG8s* is a hallmark of the response of plants to various abiotic cues. Differential expression of numerous members of *ATG8* family allows the fine-tuning of the autophagic catabolic activity of plant cells.

Autophagic degradation of plant organelles

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Autophagy, an ancient catabolic program with a primarily cytoprotective role, is emerging as a process underlying all sides of plants' life. It is triggered in response to stress, removing cellular components not vital for survival and providing energy released from degraded structures when nutrients are limited. Autophagy also occurs at a low level on a constitutive basis, assuring the turnover of 'worn-out' or damaged cell parts. It seems that autophagy is crucial for the degradation of larger cellular structures, and many organelles in plant cells have been revealed as targets of autophagy. Thus far, these include peroxisomes, ribosomes, mitochondria, endoplasmic reticulum and plastids.
We have investigated the role of macroautophagy in the degradation of peroxisomes and chloroplasts. We used stable transgenic suspension-cultured cell lines of tobacco Bright Yellow 2 that expressed a peroxisome-targeted version of Enhanced Yellow Fluorescent Protein to study the role of autophagy in peroxisome turnover in course of cultivation and ageing of the cultures. We also monitored the size of the cellular peroxisome pool, ROS production and comparative rates of organelle degradation in tobacco BY-2 cells. In leaves of *Hordeum vulgare* and *Arabidopsis thaliana*, wild type as well as *chlorina* mutants lacking chlorophyll *b*, we investigated the relationship between the stability of photosynthetic antenna complexes and the induction of autophagy. While the role of autophagy in the degradation of stromal proteins including Rubisco has long been established (Wada et al 2009 Plant Physiol), the possible role of autophagy in the degradation of components of chloroplast membranes requires further investigation.
Financial support of the Russian Science Foundation (#15-14-30008) is gratefully acknowledged. The research was done using equipment of The Core Facility Center "Cell and Molecular Technologies in Plant Science" at the Komarov Botanical Institute RAS (St.-Petersburg, Russia). Transformation of BY-2 cells was supported by funding from the Deutsche Forschungsgemeinschaft (DFG, grant number RE 1304/4-1 to SR). Part of the presented study was performed within the institutional research project no. 01201255613 of the Komarov Botanical Institute RAS.

Cytoplastic Glyceraldehyde-3-Phosphate Dehydrogenases Interact with ATG3 to Negatively Regulate Autophagy and Immunity in Plants

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The plant innate immune response includes the hypersensitive response (HR), a form of programmed cell death (PCD). PCD must be restricted to infection sites to prevent the HR from playing a pathologic rather than protective role. We find that the evolutionarily conserved autophagy pathway plays an essential role in plant innate immunity and negatively regulates PCD. Further, we show that autophagy-related protein 3 (ATG3) interacts with the cytosolic glyceraldehyde-3-phosphate dehydrogenases (GAPCs) to regulate autophagy in plants. We found that oxidative stress inhibits the interaction of ATG3 with GAPCs. Silencing of GAPCs significantly activates ATG3-dependent autophagy, while overexpression of GAPCs suppresses autophagy in *N. benthamiana* plants. Moreover, silencing of GAPCs enhances N gene-mediated cell death and plant resistance against incompatible pathogens

Tobacco mosaic virus and *Pseudomonas syringae* pv tomato DC3000, as well as compatible pathogen *P. syringae pv tabaci*. These results indicate that GAPCs have multiple functions in the regulation of autophagy, hypersensitive response, and plant innate immunity.

PCD and mechanism of gametophytic self-incompatibility in petunia

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Self-incompatibility (SI) is a pre-zygotic reproductive barrier, which prevents inbreeding in many families of angiosperms. *Petunia* possesses the Solanaceae type SI in which this reproductive barrier is regulated by the highly polymorphic *S*-locus. The *S*-locus houses the female determinant gene, *S-RNase*, and multiple male determinant genes, *S-locus F-box* (*SLF*) genes. A diploid pistil carries two different *S*-haplotypes, each producing an allelic variant of S-RNase. S-RNase is synthesized in the transmitting tissue of the style and secreted into the transmitting tract where pollen tubes grow from the stigma to the ovary. A pollen tube takes up both self S-RNase (product of the same *S*-haplotype as that carried by pollen) and non-self S-RNase (product of a different *S*-haplotype from that carried by pollen); however, only self S-RNase can inhibit the growth of the pollen tube (in the upper one-third of the style) through its RNase activity. Despite the impressive progress made in understanding the complex non-self recognition between the female determinant and multiple male determinants, there are still many aspects of S-RNase -based SI that remain unknown. It has recently been shown that programmed cell death (PCD) is triggered by SI in *Papaver rhoeas* (Bosch and Franklin-Tong, 2008). The first hint that PCD might be triggered by SI in incompatible pollen was the observation of DNA fragmentation as an important hallmark of PCD. The results of these studies contribute to our understanding of functional links between signaling components and PCD initiation in the plant cell. To elucidate whether the elimination of incompatible pollen might be associated to PCD, we applied DNA degradation analysis. To this end, petunia pistils were excised at different developmental stages, immediately frozen in liquid nitrogen and further were ground to a fine powder. Thereafter their genomic DNA was isolated by the Tri-Reagent method. Anther DNA was used as a control because it is well known that PCD occurs in the tapetum. Five micrograms of genomic DNA/lane were separated on 3% w/v agarose gel in TBE (tris-borate-EDTA) buffer at 50 v for 3 h. The evidence for DNA degradation in petunia pistils excised from flowers after pollination was obtained suggesting that this process being developed in these pistils and probably in the incompatible pollen grains. Our results demonstrated that in incompatible pollen the growth of pollen tubes is halted in the stylar area in the manner suggesting an intervention of PCD**.** In addition, based on the available laboratory data on ethylene involvement in the regulation of petunia male gametophyte development, germination and growth in the course of the progamic phase of fertilization, the hypothesis on ethylene participation in functioning of the SI mechanism including PCD induction was put forward.

SESSION 6

Complex Adaptive and Neuron-Like Reactions

A novel control module for thermomemory in plants

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Acquired thermotolerance is an increased resistance of cells, tissues and organisms to elevated temperature following a prior exposure to heat (thermopriming). Several studies provided evidence that plants maintain their acquired tolerance for a period of time (memory); hence, they are able to modify their responses and thus perform more efficiently upon future severe stresses. However, the molecular mechanisms underlying thermopriming, and in particular thermomemory, are currently not well understood.
To identify cellular determinants of thermopriming and -memory in plants, we screened *Arabidopsis thaliana* accessions for their survival rate after a priming and triggering heat stress and identified N13 as a strong thermomemory accession, while Col-0 turned out to have a weak thermomemory. We employed a combined pharmacological/genomics approach to identify molecular determinants of thermomemory and discovered that differences in the accumulation of the small chloroplast-localised heat shock protein HSP21 strongly contribute to the differential thermomemory performance of N13 and Col-0. Rapid degradation of HSP21 protein during the memory phase due to proteolytic cleavage limits thermomemory (Col-0), while extended persistence of HSP21 promotes memory (N13), indicating an important role of plastid-localized proteases for establishing memory after a priming heat stress. In fact, we identified a plastid-localised metalloprotease, FtsH6, for which no previous *in vivo* function was reported, as a protease involved in the initial degradation of HSP21 during the memory phase in Col-0, while FtsH6 is non-functional in N13 allowing HSP21 protein to remain accumulated during the memory phase. Our results thus reveal the presence of a plastidial protease - HSP21 control module for thermomemory in plants.

Within leaf variation is the largest source of variation in agroinfiltration of *Nicotiana benthamiana*

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Syringe agroinfiltration offers a simple and efficient technique for transgenic applications. Leaves of *Nicotiana benthamiana* show reliable and high transformation efficiency, but in quantitative assays have a certain degree of variation. We used a nested design in our agroinfiltration experiments to dissect the sources of this variation.
Firefly luciferase gene, containing an intron, was used as a reporter for agroinfiltration. Top leaves of six week old tobacco plants were infiltrated, several samples were punched from each leaf after two days of transient expression, and protein extracts from the samples were repeatedly measured for luciferase activity. Interestingly, the sampling spots in the leaves showed most of the variation although the bacteria were evenly distributed in the infiltrated leaves. The next important source was the different leaves on each plant. Variation between experiments, between plants and between repetitive measurements of the extracts could be easily minimized.
Efforts and expenditure of agroinfiltration experiments can be optimized when sources of variation are known.

Electrical impedance spectroscopy in halophytes

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In order to evaluate the applicability of the electrical impedance spectroscopy method in halophytes (naturally salt tolerant plants), electrical impedance spectra were compared in the leaves of two coastal halophytes (Cakile maritima and Zygophyllum album) cultivated under different growth conditions (biotope versus controlled conditions, hydroponic versus sand system cultures) and different salt stress conditions. The kinetic of impedance parameters was also monitored under short term salinity. The spectra of electrical impedance of leaves under biotope and laboratory conditions showed difference in the electrical response of *Cakile maritima* in the biotope and laboratory conditions. The response of electrical impedance parameters to salinity was also different in the hydroponic system when compared to the soil one, indicating more stressful conditions in solution culture. The amplitude of the curves of impedance spectrometry decreased when plants were stressed comparatively to their controls, with the highest electrical resistance in the presence of 50 and 100 mM while the lowest value was at 400 mM NaCl. The electrical resistance increased at an early stage after the application of salt stress reaching maximal value 180 min later, before it rapidly declined thereafter. The observed peak can translate a signal, that the plant could have received, which triggers a cascade of metabolic reactions allowing the plant to regain its hydro-ionic balance. In conclusion, electrical impedance spectroscopy can be used to quickly compare different growth conditions as well as different salinity treatments. This method can also separate between the osmotic and the ionic phases of the response to salt stress.

Cell mechanisms of adaptation to salt stress on the level of cell culture

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Since the 70s of the last century it was established that the one of the promising approaches in the study of mechanisms of salt tolerance in plants is the use of cell and tissue culture methods (Strogonov et al, 1970). This approach is relevant now because cultured tissues have greater plasticity and adaptability to salt stress compared with origin intact plants. This may be associated with features of cytodifferentiation and intercellular communications, which include exchange by biologically active signal molecules. Due to insufficient knowledge of these issues at the level of cell and tissue culture, we have set the objectives to find out regularities of morphogenesis and cellular differentiation and to figure out histo- and cytochemical features of cultured tissues in the process of adaptation to salt stress.

As a result of screening more than 20 varieties and perspective wheat lines on the level of seedlings and seeds the varieties which contrast in salt resistance have been selected and used as objects for study of the salt resistance mechanisms at the level of cultured cells. The effect of salt stress (0.1%, 0.25%, 0.5%, 0.75%, 1.0%, 1.68% NaCI) on the processes of morphogenesis and cell differentiation *in vitro* of genotypes which contrast in NaCI resistance has been studied. In the resistant variety Kazakhstan-10 we found increased growth and embryogenic potential of tissues under the influence of concentration 0.5% NaCI, where in the salt sensitive varieties inhibition of growth and morphogenesis of calli were observed. It was found, that the distinctive features of salt tolerant calli of genotypes resistant to NaCI are the decrease of callus cells' length, the appearance of embryogenic cells of spherical shape, the initiation of embryos, the appearance of cells with signs of programmed cell death (PCD) or apoptosis. Extracellular polysaccharides and proteins released by cells with signs of PCD in the process of adaptation of resistant varieties' tissues to stress have been identified by histochemical and cytochemical methods.

In general, one of the possible cell mechanisms of adaptation to salt stress in the *in vitro* system has been identified: part of cells population degrades by PCD and releases extracellular substances of polysaccharide and protein nature during the death. These substances may have a protective and a growth-regulatory effects by stimulating the synthesis of protective callose coat in neighboring cells and by the formation of cells which competent to embryodogenesis. Also they inhibit the cells' growth by elongation and could switch their developmental programm on the path of mitotic divisions. All this

may lead to the increased growth and embryogenic potential of resistant genotype' callus tissues under the salt stress.
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Exploring the metabolome of higher plant species

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The biochemical diversity in the plant kingdom is estimated to well exceed 100,000 distinct compounds. Although our knowledge of the enzymes and regulatory genes working in a complex network to generate this large arsenal of metabolites is gradually increasing, direct experimental evidence for molecular function in metabolite biosynthesis is available for a mere handful of genes. In recent years, extended genomic resources and high-throughput DNA sequencing methods enabled the full genome sequencing of a large number of plant species, as well as large-scale sequencing of a comprehensive panel of accessions (of a single plant species). This, together with modern metabolomics approaches, allows conducting genome-wide association studies (GWAS) with metabolic traits for the identification of metabolic quantitative trait loci (mQTLs). We are currently performing GWAS in Arabidopsis, maize and rice in order to identify mQTLs. To do so, we are first preforming metabolic-phenotypic characterization of the metabolites (primary, secondary and lipids) in different accessions of those species, using gas- and liquid-chromatography mass-spectrometry (GC-MS and LC-MS) analysis. This allows the simultaneous identification of a large number of genetic loci controlling the metabolome. For selecting candidate causal genes in a given locus, we first search for sequence homology between genes in the locus and previously characterized genes; we compute in addition the linkage disequilibrium between SNPs in the locus. Another approach we are taking for causal-gene identification makes use of the advent of multi-parallel integrative methods for the measurement of gene expression and metabolite abundances in order to construct gene-metabolite networks. Finally, candidate genes are validated using transgenic plant approaches. Advantages of metabolomic profiling and examples illustrating the possibilities of using metabolomic approaches to answer diverse biological questions will be discussed.

Guidance of circumnutation of climbing bean stems: An ecological exploration

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Living organisms guide the movement of effector organs or cells in response to stimuli to make, or avoid, contact with things—be it a bee flitting from flower to flower, a gibbon swinging from branch to branch, or a peregrine falcon diving on a flying pigeon. Strikingly enough, plants, unlike animals, are commonly believed to remain still, with their behavioral repertoire reducing to invariant tropistic or nastic responses implemented in the form of sets of fixed reflexes. The need to control their movements is thereby eliminated or seriously undermined. And yet plants are as much in the move as any other living organism.
In this report, we consider the possibility that the power of movement in plants, to echo Darwin and his son's seminal work, is not forced, or hardwired, but rather appropriately controlled as much as the movements performed by bees, gibbons or peregrine falcons are. More specifically, we shall focus our attention on what is probably the simplest form in which general circumnutation can be modified: the one exhibited by twining plants, in particular, by common beans (*Phaseolus vulgaris*) as they approach and twine spirally round supports. Unlike leaf-climbers, tendril-bearers, and hook and root climbers, bean shoots rely on an increase in the amplitude of an otherwise ordinary movement of circumnutation. Such basic, and yet modified, revolving nutation shall be the focus of our attention. In particular, we aim to explore the guidance of circumnutation of climbing bean stems under ecological principles.
In this talk we present some preliminary results on the control of circumnutation by climbing beans, and explore the possibility that the power of movement in plants, more generally, is controlled under ecological principles. The underlying idea that motivates this research is the suspicion that the control of movement in plants is not unlike the control of movement in animals. Plants and animals, we contend, have functionally similar internal systems for organizing sets of behaviors. In essence, a plant

that orients towards, say, a source of energy behaves in functionally the same way as an animal that runs towards its prey. It is in this sense that the type of control required to perform such actions is our object of study.
With that being said, that plants or animals control their movements does not imply that their behavior is to be accounted for in computational or information-processing terms. In fact, our working hypothesis is that both plants and animals guide their movements ecologically—non-computationally. According to ecological psychology, plants, like animals, perceive what is available in terms of biologically relevant interactions. Climbing plants are in this way ecological perceivers. Vines perceive possibilities for action, such as when a support is perceived as affording climbing.
Under this framework, the proper unit of analysis is the whole organism-environment system as such. A climbing plant and its support constitute an ecologically coupled system in which the action of twining and the perception of affordances form a continuous and cyclic loop. Despite things being in constant flux, some relations remain unchanged, and organisms can pick them up. This information is relational, and takes the form of invariant properties of the underlying structure of an ever-changing environment that can in principle be directly detected. Ecological psychologists say that environmental information is *specificational*: information in the vicinity of a climber specifies ways for the plant to interact with features, such as the support standing nearby. Our working hypothesis is that plants, like animals, pick up the invariant structure of an ever-changing environment.
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Plant polysaccharide-based "muscles": features of organization and occurrence in the plant world

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Plant "muscles" is the name reflecting the action mode of specialized cells of plant mechanical tissue - gelatinous fibers. They have important functions for the plant, since they can generate high tensional stress within mature organs, thus either enabling the movement of these organs or reinforcing their structure and stability. Gelatinous fibers have exceptional length of several centimeters, attainable by intrusive growth, and thickened cell wall. In addition to the primary and secondary cell wall gelatinous fibers capable to formation of tertiary cell wall (gelatinous), which is characterized by high content of axially oriented cellulose and presence of rhamnogalacturonan I as basic polysaccharide of matrix. It is the presence of this pectin component and plays a crucial role in the ability of gelatinous fibers to develop contractile properties.
Gelatinous fibers are found in various plant organs, including roots, stems, petioles, tendrils, peduncles, thorns and contractile roots. They occur in phloem and xylem of both primary and secondary origin, and sometimes in non-vascular tissues. Gelatinous fibres have been observed in diverse taxa of higher plants, including the evolutionary early orders of vascular plants. Thus, the G-fibres may have evolved in an early stage of land plant evolution.
The report will discuss the biogenesis of gelatinous fibers and the organization of their cell wall providing the formation of contractile properties. Gelatinous fibers occurrence in the plant world as well as modification of their cell wall organization in plants of different taxa will be considered.
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Location of signaling molecules in common regulatory system of cells, organs, organisms

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The report presents and gives scientific credence to the conception of a trigger mechanism engaging signaling molecules into metabolic regulation and based on selectively changed direction or intensity of a substance flow in stationary biochemical reactions in cells, organs or systems. The core principles of the conception are as follows.
1. Any biological system is an open system. All the internal changes are caused by the changing external existence conditions.
2. Most of trigger mechanisms for regulatory process in a cell, organ or system are performed at the epigenomic level, and the variety of choices for the regulation is vastly enormous.
3. Metabolic disturbance which happens in one cell may launch a chain reaction through the changes in metabolic flows and lead to imbalance in other organs and tissues which are relevant to the changes in particular mass metabolic flow; signaling molecules are involved in the process as well. Thus, signaling molecules are enabled by changing the mass transport of a substance caused by some internal or external factor. Internal factor in this context is considered to be ontogenetic DNA-reading.
4. The regulatory mechanisms of general biological coordination in cells, tissues and organs often depend on different levels of gene expression, forming quick responses.
5. The respective signaling molecules are in charge of certain places and some biochemical reactions in order to start or quit the processes, but the very signaling molecules are produced because of some changes induced by certain factors.
The data to support this conception are presented.

Nanoparticles induce signalling reactions and affect physiological processes in *Arabidopsis thaliana* plants

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Nanoparticles (NPs) have gained particular attention from industrialists due to their relatively low cost of production and tremendously enhanced physical/chemical characteristics. Silver nanoparticles (Ag NPs) are the world's most important nanomaterial. Nearly 25% of all nanotechnology consumer products include Ag NPs. The dramatic increase in industrial use of Ag NPs has raised considerable concern about their potential release and effects on flora and ecosystems, as well as the possibility of it entering human food chain through plants. The soil concentration of Ag NPs in agricultural land (estimates for USA, 2012) exponentially increases, mainly due to the treatment with Ag-NP-polluted sludge, with the average predicted concentration of Ag NPs in the soil of approximately 8 mg kg^{-1} (Gottschalk *et al*., 2013, Environ Pollut). This is an average number while "hotspots" contain much more nanosilver. Concentrations of Ag NPs in surface water and sewage treatment have been increasing significantly in recent years.
In this work, we have aimed to establish the pattern of regulatory and stress reactions of manufactured Ag NPs in model plant *Arabidopsis thaliana*, at the organismal level (root and leaf growth) and at the cellular level (Ca^{2+} signalling, ROS generation, plasma membrane conductances, photosynthetic efficiency). We also examined the Ag accumulation in higher plants cultivated on Ag NP-containing media, and the potential action of Ag NPs on extracellular L-ascorbic acid.
We have shown that addition of Ag NPs to cultivation medium, at levels above 300 mg L^{-1}, inhibited *Arabidopsis thaliana* root elongation and leaf expansion. This also resulted in decreased photosynthetic efficiency and extreme accumulation of Ag in tissues. Acute application of Ag NPs induced transient elevation of $[Ca^{2+}]_{cyt}$, and accumulation of ROS. Whole-cell patch-clamp measurements on root cell protoplasts demonstrated that Ag NPs slightly inhibited plasma membrane K^+ efflux and Ca^{2+} influx

currents or caused membrane breakdown. However, in excised outside-out patches, Ag NPs activated Gd^{3+}-sensitive Ca^{2+} influx channels with unitary conductance of approximately 56 pS. Bulk particles did not modify the plasma membrane currents. Tests with electron paramagnetic resonance spectroscopy showed that Ag NPs were not able to catalyse hydroxyl radical generation but they directly oxidised the major plant antioxidant, L-ascorbic acid.
Overall, the presented data sheds the light on mechanisms of the impact of nanosilver on plant cells and show that these include induction of classical stress signalling reactions (mediated by $[Ca^{2+}]_{cyt}$ and ROS) and a specific effect on the plasma membrane conductance and the reduced ascorbate.
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Regulation of plant fiber development: RNA-seq snapshot of transcription factors

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Higher plant organism consists of around 40 functionally different cell types. Their specialization is among the major processes of plant ontogenesis. The study of a plant cell functional specialization is usually hampered by the difficulty or even impossibility to isolate plant cells of certain type and at certain stage of development in the quantities sufficient for in-depth characterization. Since plant samples usually contain many different cell types, it is difficult to characterize tissue (cell)-specific processes. One of the rare experimental systems that permit(s) to study specialization of certain cell type *in planta* is developing primary phloem fibers of flax (*Linum usitatissimum* L.). The major function of mature flax phloem fibers is the provision of strength and flexibility to high and narrow stem, which experiences severe mechanical stresses.
Plant fibers are the most widely spread type of cells in mechanical tissues in the vegetative organs of the terrestrial vascular plants. Two key processes have the major impact on the specialization of the plant fibers: intrusive growth and the cell wall thickening. These two stages are distinctly separated in time and space, allowing to analyze tissue and stage-specific components. At advanced stage of specialization the fibers of many plant species form a special type of the cell wall - fiber-specific cellulose-enriched cell wall type, or a tertiary cell wall (TCW). Unlike the secondary cell wall (SCW), that consists of cellulose, hemicelluloses and lignin, TCW is rich in cellulose (up to 90%) and lacks detectable xylan and lignin. The synthesis of TCW always begins after deposition of at least one layer of SCW. The molecular events associated with the activation and regulation of gene expression during two key stages - the elongation growth and the tertiary cell wall synthesis - have not been studied at all yet.
We performed large-scale transcriptional profiling of the fibers, taken at different development stages. Due to the compact (within bundles) location in stem and presence of thick cell wall, phloem fibers at advanced stage of specialization can be effectively purified from the surrounding tissues and used for various types of analysis. After sequencing using the platform Illumina, about 95% of single-end reads (75bp) were successful mapped to the reference sequence using the Cufflinks protocol. In total, expression of 36012 genes was detected across all samples, while whole-genome assembly of flax contains 43484 protein-coding genes (Wang *et al*., 2012). Results of analysis using the algorithm CuffDiff have indicated that 1124 genes had statistically significant differences in expression level at values of $q<0,05$ at least in one pair of the analyzed samples.
Out of 2481 genes of transcription factors indentified in flax genome and classified into 57 families (Jin *et al*., 2014), 2027 were detected in our experiment. Transcriptome profiling revealed that the expression of secondary wall NAC (NST1, SND1) and MYB (MYB46, 83, 20, 52, 63, 103 and others) transcription factors activating and regulating the SCW formation (Zhong et al., 2010; Hussey et al., 2013; Didi et al., 2015) is absent or greatly reduced in fibers at advanced stage of specialization compared with other tissues or fibers at stage of intrusive growth. Also we detected considerably lowered level of transcript abundance in fibers with tertiary cell wall for all known genes that are ascribed to xylan synthesis and for most of those involved in phenylpropanoid metabolism leading to lignin formation. At the same time in fibers, the most abundantly expressed genes coding bZIP, CO-like, HD-ZIP, MYB-related, NAC and TCP families. This indicated that the initiation and control of TCW synthesis are likely associated with the activation of other transcription factors, candidates for which are identified in our study.
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The effect of copper nanoparticles on growth, cell viability and signaling processes of wheat plants

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Introduction. Copper nanoparticles (Cu NPs) are very important nanomaterial. The use of Cu NPs in industry progressively increases while their effects on living systems remains poorly understood. Copper is important micronutrient but it is also a toxic heavy metal. It is unknown how the solid form of copper will react with biosystems such as plant cells.
The aim of this study was to disclose effects of Cu NPs on growth, cell viability, PCD and ROS generation of wheat plants (*Triticum aestivum* L.).
Materials and methods. Certified Cu NPs (~40 nm diameter) were from American Elements and were tested by Electron microscopy. Plants (Vasilisa cultivar) were from annotated collection of Jodzina National Agricultural Institute (Belarus). 1-500 mg L^{-1} Cu NPs were prepared in water buffer solutions as suspensions by ultrasonication. They were freshly prepared and instantaneously applied directly to plants or to cultivation media. To study the effect of Cu NPs on the wheat embryo, they were prepared from the field-grown plants at the appropriate developmental stage (in the end of June). Sterile embryo culture was generated and maintained during experiment. Growth and cell biology testing was carried out using hydroponically grown wheat seedlings (4-7-day old).
Results. Application of Cu NPs to tips of roots inhibited their elongation starting from 10 mg L^{-1}. 200-500 mg L^{-1} of Cu NPs completely inhibited root and leaf growth. 10-30 mg L^{-1} of Cu NPs significantly inhibited growth and formation of callus in cultivated wheat embryos. Cell viability and morphology tests demonstrated that Cu NPs induced typical symptoms of programmed cell death (PCD) in root hairs. Chronic application of Cu NPs resulted in activation of cell death proteases in root hairs as measured using CASPACE® FITC-VAD-fmk kit. Moreover, Cu NPs significantly stimulated generation of superoxide radical (test with fluorescent probe dihydroethidium). EPR spectroscopy experiments demonstrated that Cu NPs were capable of catalysing Fenton-like generation of hydroxyl radicals. Hypothetically, this can be a reason of observed physiological effects.
Conclusions. Our data showed that Cu NPs are toxic for wheat in very low concentrations and that this toxicity is probably related to death of root hairs through hydroxyl-driven apoptosis-like PCD.

The role of tubulin and actin cytoskeleton rearrangements during pea (*Pisum sativum* L.) symbiotic nodule development

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Cytoskeleton rearrangements have a crucial role in nodule organogenesis and functioning. However, only a limited number of studies are available on the role and structure of the cytoskeleton during nodule development. The pea symbiotic nodule is a useful model to study the molecular and cellular mechanisms of the legume-*Rhizobium* symbiosis.
Using immunocytochemical analysis and confocal laser scanning microscopy, the three-dimensional organization of microtubules and actin microfilaments in each histological zone in pea nodule was analyzed and linked to the developmental processes during nodule cell differentiation. The pea wild-type SGE (Kosterin & Rozov, 1993) and corresponding mutant lines SGEFix$^-$-1 (*sym40*), SGEFix$^-$-2 (*sym33*) (Tsyganov et al., 1998) were used. The *Sym40* gene is orthologous to the *M. truncatula EFD* gene (Nemankin, 2011). The *Sym33* gene is orthologous to the *M. truncatula IPD3* gene (Ovchinnikova et al., 2011).
It has been revealed the important role of endoplasmic microtubules and actin microfilaments 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. The alteration in orientation of actin microfilaments after bacterial release was not observed. High dense network around nuclei in different types of nodule cells is the particularity of actin cytoskeleton.
Thus, it seems that tubulin and actin cytoskeleton can function at different steps of nodule cell differentiation.
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Molecular control of flowering in strawberries

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Strawberries are grown on all habitable continents. Many strawberry growing areas are bound to be affected by the progressing climate change, which may cause problems for the flowering of strawberries, and subsequently reduce yield. To meet the environmental challenges, plant breeders need tools for developing new cultivars that are suitable for growing in specific environments. Although studying flowering responses directly in the cultivated strawberry could provide immediate practical applications, it is hampered by the complex genomics of the species.
The woodland strawberry *Fragaria vesca* (L.) has arisen as a convenient model plant for strawberries. It is a diploid species and therefore has a less complex genome than the cultivated octoploid strawberry. It is also amenable to genetic transformation and has a short life span of approximately three months.
In woodland strawberry, a locus named the *SEASONAL FLOWERING LOCUS* (*SFL*) is known to control the switch from seasonal to continuous flowering habit. Therefore, elucidation of the molecular pathways controlling flowering in strawberries was begun by identifying the molecular nature of the *SFL*. Via genetic mapping, *SFL* was identified as the woodland strawberry orthologue of *TERMINAL FLOWER1* (*FvTFL1*). *TFL1* in *Arabidopsis* has been shown to be a strong floral repressor. In woodland strawberry, *FvTFL1* was shown to be photoperiodically regulated, and the continuous flowering habit was shown to be caused by a mutation at *FvTFL1*, leading to a defective protein product. The role of *FvTFL1* as a floral repressor was confirmed by experiments with transgenic plants.
We have also studied the exceptional flowering response of the subarctic *F. vesca* accession 'Alta'. This accession appears to have an obligatory requirement for vernalization, as it does not flower even after prolonged exposure to short days. Studying the expression pattern of *FvTFL1* in 'Alta' showed that in this accession *FvTFL1* is actually upregulated by short days and very low temperature is required for downregulating the gene.
The findings on *FvTFL1* were extended to cultivated strawberry. It was demonstrated that silencing the *F.* ×*ananassa* homologue of *TFL1* (*FaTFL1*) resulted in early flowering, and that differences in the regulation of *FaTFL1* were associated with different flowering times in strawberry cultivars. The finding that *FaTFL1* is a major determinant in the flowering response of cultivated strawberry provides breeders with a new breeding target; producing cultivars with lowered *FaTFL1* expression level could expand the flowering and fruiting season of strawberries.

Expression maps of PINs transporters in the root meristem of *Arabidopsis thaliana*

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In the root meristem, the PIN proteins are the key mediators of polar auxin transport acting in a cell as the efflux carriers. Separated facts about PINs expression are dispersed across a number of papers related to the auxin pathway. Integration of these puzzle pieces about PINs expression revealed that, along with a recurring pattern, some features of PINs expression varied from article to article. To determine if this uncertainty has a basis in the variability of PINs gene activity, we performed comprehensive 3D analysis of PINs expression patterns in *Arabidopsis thaliana* roots.
The roots of 3 dag seedling of *Arabidopsis thaliana* Col-0 (L.) were double-labelled by the specific PINs antibodies. As a result of this analysis, we provide the detailed maps of PIN1, PIN2, PIN4 and PIN7 expression in the root meristem.
The variability in *PIN* expression pattern observed in individual roots may occur upon the differences in auxin distribution among plants. To imitate this effect we analyzed *PIN* expression in the roots from the seedlings treated with different auxin concentrations and *pin* mutants. For example, we showed that the changes in *PIN1* expression after auxin treatment and in *pin* mutants mainly fit to the spectrum of variability in wild type (Omelyanchuk et.al., 2016).
Our results suggest that PINs expression patterns in the root meristem reflects well the changes in auxin distribution.
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Phototropin responses: Getting from intracellular biology to organ-level behavior, adaptation and fitness

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Plants are exquisitely sensitive to changes in their environment and have evolved a myriad of sensors and associated signaling pathways that mediate adaptive responses. Though environmental signals are perceived at the level of single proteins, such sensing needs to be integrated into cellular, tissue, organ and ultimately whole plant physiology. We will report on our studies of the blue light photoreceptor, phototropin 1 (phot1), and its ability to mediate just these types of integration. For example, upon sensing changes in directional blue light, phot1 rapidly reorganizes its intracellular localization and initiates intra- and intercellular signaling. These events appear to involved posttranslational modifications such as phosphorylation and ubiquitination. It appears that these modifications result in distinct 'pools' of phot1, only some of which lead to further physiological signaling. Phot1 does not operate in isolation to alter physiology: phot1 works in concert, through genetic and direct physical interactions, with a variety of molecules to properly integrate multiple signals impinging on the plant at any given time. These interactions include ones with other photoreceptor molecules, such as phytochrome A, as well as with proteins as seemingly unconnected as ones involved in plant pathogen signal-response systems. Ultimately, all of these signal integration events and convergence of physiology lead to adaptive biology that ensure fitness of the plant in the natural environment. Our studies have begun to elucidate some of the fitness consequences of normal phot1 signaling and suggest some direct applications of such knowledge to plant-based industries.

Universality in root behaviour

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Starting from the original Kleiber's observation that, for the vast majority of living organisms, the metabolic rate scales to the 3/4 power of their mass, many variables in life were found to obey such scaling law. In biology the law is typically a simple power law: X = aMb where X is something measurable (e.g. metabolic rate, growth rate, tree height), a is a constant, M is the mass of the organism and b is an exponent that in an interesting way almost always approximate a multiple of ¼. Scaling laws as a consequence of generic processes have been extremely useful in helping scientists to gain comprehensions of many different phenomena.
Despite the astonishing variety and complexity of the root apparatus in plants, some universal scaling laws can be demonstrated also in roots. Their significance in terms of plant behaviour will be discussed.

Plants responses to light mechanical stimuli with ecological implications

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Mechanical stimuli is one of the most common stress factor which may affect new adjustments in plant growth and development. The broader ecological significance of induced plant responses to mechanical stimuli on behavior of herbivore insects and their natural enemies has not been explicitly investigated. To identify effects of plants respond to brief touch on pattern of biomass allocation and tritrophic interactions we used model system consisting of monocotyledon plants (maize) and dicotyledon plants (bean), their most common pests *Rhopalosiphum padi* L. and *Aphis fabae* Scop. and natural enemies of pests ladybirds, *Coccinela septempunctata* L. Brief and light mechano stimuli, one minute per day in six days, significantly reduced stem height and specific leaf area in both plant species. Maize plants allocated more biomass to the roots while bean invested more biomass into above ground parts. Significant increases in volatile emission of (E)-nerolidol and (E)-β-caryophyllene in maize and 6-methyl-5-hepten-2-one and an unidentified sesquiterpene in bean show that touching had systemic effect in both plant species. The changes in volatile emissions induced by brief and light mechano stimuli made plants significantly less attractive to aphid pest species compared to untouched controls. Ladybirds showed also avoidance reposes to touched plants. Our study shows that light and brief mechanical stimuli can induce considerable changes in plants which have potential to affect host plant selection and acceptance by aphids and habitat searching by ladybirds. The link between plant response to mechanical stimuli and insect behaviour identified in our study represents a new phenomenon that contributes to the broader ecological significance of induced plant responses to mechanical stress.

Phytochrome control of underground diatropic shoots – stolon and rhizome growth orientation

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Light is critical for plants because they dependent on it as a source energy and information. Light signals provide information of crucial ecological value at many development stages. The three classes of plant signal-transducing photoreceptors – phytochromes, cryptochromes and phototropin. The phytochromes (Phy) are signal transducing photoreceptors that convert between inactive and active forms in response to different wavelengths of light. Phy are involved in the perception of red (R) and far-red light (FR), two other photoreceptors, cryptochromes and phototropins, have been identified which are associated with ultraviolet-A wavelengths. Phy A to E are red-light and far-red-light photoreceptors that secondarily also absorb blue light. In higher plants, unilateral red light induces weak

positive phototropism in the root of *Arabidopsis* (Ruppel et al. 2001, Kiss et al. 2003). Whereas shoots bend toward the direction of incoming blue light, improving the chances of light-harvesting organs to collect light for photosynthesis, root bend away from the direction of incoming blue light stimuli, avoiding the stressful conditions of upper soil layers (Esmon et al. 2005). Depending on soil type, light can penetrate to a depth from 1 to 3-5 cm (Tester and Morris 1987).
Shoots grow toward light, exhibiting positive phototropism and negative gravitropism. Roots exhibit positive gravitropism and negative phototropism, growing toward the gravity vector. There are, however, numerous exceptions like underground horizontally growing (hypogeodiagravitropic) shoots as stolons and rhizomes. Diagravitropism – growth of plant part transversely to the Earth's gravitation centerline. Physiological mechanisms of rhizome and stolon diagravitropism are very limited. Experimentally, the phytochrome-dependent growth responses of rhizomes and stolons were studied to reveal the role of phytochrome in controlling underground diatropic growth (Markarov and Golovko 1995). Red-light or white-light irradiation induces a negative phototropic response in the apical zones of rhizomes and stolons whereby far-red light reverses the red-light effect. Therefore, Phy R supports plagiotropic growth of rhizomes and stolons under soil surface and Phy FR prevents the shoot apex appearance. Phytochrome control on diatropic growth orientation is effective till the beginning of leaf primordia formation. Diagravitropism of rhizomes transforms into negative gravitropism in autumn and underground buds produce orthotropic shoots in spring after period of dormancy. Unlike rhizomes and stolons, underground shoots if they produce sarments are capable of changing diatropic (horizontal) growth to orthotropic (vertical) growth without any period of dormancy (Markarov 1996, Maslova et al. 2006).
Now we have some progress in knowledge about mechanisms of root and shoot phototropism. Participation of various photoreceptors and their interaction in regulation of these processes is shown. The role of Phy in phototropism of *Arabidopsis thaliana* shoots and roots is studied (Ruppel et al., 2001; Lariguet et al., 2003, 2006; Kiss et al., 2003; Correl, Kiss, 2005; Kumar et.al., 2008; Hopkins, Kiss, 2012). At present, physiological and molecular mechanisms of diagravitropism are still understudied. Nothing is known about molecular mechanisms of morphogenesis for diagravitropic shoots and phytochrome regulation of stolon and rhizome phototropism and gravitropism. Data on specific genes and proteins involved in rhizome differentiation, development, and functions are single (Kaur et al., 2008; Ruifeng et al., 2012). Presence of photosynthesis genes including photosystem I and II polypeptide, chlorophyll *a*, apoprotein A1, phototropin-2 in rhizome elongation zone is a proven fact. Expression of such genes can be in the photophilous response period of rhizome apical zone under light expose. In general, the problem of photomorphogenesis regulation and growth orientations of underground shoots is of a practical importance as light spectral structure is one of the major factors impacting intensity of shoots formation, productivity and resistance of cenosis.

Glutamine-dependent coupling of metabolism and signaling in *Chlorella*

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All photosynthetic organisms require nitrogen for optimum growth. However, nitrate/nitrite is not used by *Chlorella variabilis* NC64A. Moreover, ammonium does not play the same central role in this alga as it does in *Chlamydomonas reinhardtii* or in many bacteria. According to our data, glutamine is the preferred nitrogen source for *C.variabilis* strain NC64A.
Glutamine is a metabolite of central importance in nitrogen metabolism of microorganisms and plants. When nitrogen is abundant, oxygenic phototrophic organisms, from cyanobacteria to higher plants, are suggested to store nitrogen as arginine, by relieving feedback inhibition of the arginine biosynthesis controlling enzyme, N-acetyl-L-glutamate kinase (NAGK). Our results provide crucial information for sequence and transcriptional analysis of Cv*GLB1* gene encoding PII in *C. variabilis* NC64A (CvPII). The coding region of the Cv*GLB1* gene was sequenced, and it contained six introns and seven exons. In comparison to PII proteins from plants and cyanobacteria, the predicted amino acid sequence showed its highest degree of identity with proteins from *Chlamydomonas* (55.2 %) and cyanobacteria, *Synechococcus PCC 7942* (52.3%) and *Synechocystissp PCC 6803* (51.8%). Expression analysis indicates that Cv*GLB1* transcription is independent on growth rates or nitrogen availability, suggesting that it may be used as a reference gene. We also demonstrated that CvPII shares high sequence identity with PII proteins from plants and cyanobacteria.

CvPII forms a trimer and CvNAGK is a hexamer, and together with the conservation of the residues involved in molecule interacting, this confirms that CvPII functions as signaling protein. The C-terminus of CvPII contains a similar sequence than the C-terminus of PII from *C.reinhardtii*, which was shown to bind glutamine and to mediate glutamine sensing. In PII, the C-terminal extension forms a small loop structure, the Q-loop (KMEG) that wraps around the bound glutamine molecule and is required for glutamine-dependent complex formation with N-acetyl-L-glutamate kinase. The present study shows that CvPII controls, in a glutamine-dependent manner, the key enzyme of the ornithine/arginine biosynthesis pathway CvNAGK that leads to arginine formation. To our data externally supplied glutamine directly influences the internal pool of arginine in NC64A.
Glutamine synthetase (GS) catalyzes the ATP-dependent conversion of glutamate and ammonium to glutamine. The total GS activity measurements showed that the values of enzyme activities correlated negatively with glutamine levels in the media. These data emphasize the importance of glutamine-dependent coupling of metabolism and signaling as components of an efficient pathway allowing the maintenance of metabolic homeostasis and sustaining growth of *Chlorella*.
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The control of growth plasticity by NAC transcription factors

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Environmental perturbation affects key processes of plant physiology and development and transcription factor (TF)-controlled expressional reprogramming plays an important component in this response. NAC transcription factors represent one of the largest TF families in plants, with generally more than 100 members in each species. The functions of many NACs have been identified in recent years, in *Arabidopsis thaliana* and other plants, and increasingly the details of the gene regulatory networks they control are unveiled. Here, we report the identification and functional characterization of a NAC TF from Arabidopsis, tentatively called RAF, which controls root development upon ABA treatment and during osmotic stress. RAF suppresses the formation of root hairs and inhibits primary and lateral root growth, typical characteristics of drought rhizogenesis, an adaptive response of the root to drought stress. RAF represses several genes important for root hair development, including RSL4. Another NAC with a major impact on plant growth and the response to abiotic stress is JUNGBRUNNEN1 (JUB1), which in German stands for "Fountain of Youth", named after its property to extend plant life span when overexpressed. JUB1 has a dual function: it directly activates genes improving the tolerance to abiotic stress (such as *DREB2A*, *HSFA2* and *MBF1c*), but also directly represses the activity of genes involved in the biosynthesis of the growth hormones GA (gibberellins) and BR (brassinosteroids). *JUB1* itself is under the repressive control of the TFs PIF4 and BZR1, adding a further layer of control to the complex regulatory network that involves JUB1. New results will be presented.

Auxins and Indoleamines: Signaling in Plant Regeneration

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The indoleamines melatonin (MEL: N-acetyl-5methoxytryptamine) and serotonin (5-HT: 5-hydroxytryptamine) are neurological signaling molecules with diverse roles in plants. Both MEL and 5-HT are synthesized from indoleacetic acid and tryptophan in higher plants and the various metabolic roles are frequently related to auxin metabolism. Recent studies have shown that MEL promotes root development with specific increases in lateral and adventitious rooting. In some cases, MEL inhibits primary root growth while simultaneously increasing lateral root differentiation. Likewise, 5-HT has been shown to interfere with auxin transport or to redirect auxin stimulation to induce lateral rooting. The reallocation of plant resources in response to the indoleamines may include modification of sugar metabolism. Both MEL and 5-HT interact with the established plant signaling networks, especially

auxin signaling, to redirect plant resources during explant stress and de novo organogenesis. The objectives of our studies were to determine the mechanisms of plant regeneration in response to MEL and 5-HT. Dark grown tissues contain higher concentrations of MEL and are more competent to regenerate. Excising the explant separates competent cells from the maternal tissues thereby disrupting cell-to-cell communication and applying a mechanical stress that induces ethylene and auxin. The accumulation of auxin at the cut surface results in undifferentiated cell growth and/or proliferation of competent cells. Calcium signaling between cells at the cut surface selects the cells that undergo differentiation and organogenesis. Inhibition of MEL inhibits de novo root organogenesis. Overall, auxin, indoleamines, and calcium form a signaling cascade that directs plant growth and development.

Precipitation and air temperature affect plant communication and defense

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Plants can perceive a multitude of cues and signals from their environment, including neighboring plants and herbivores. As a response, they generate specific phenotypes or show different behavioral strategies allowing them to gather resources, compete, or defend. For example, previous work showed that competition towards kin neighbors in *Pisum sativum* depends on the nutrient content in the soil. In this work, we investigated if water availability could affect volatile communication and defense in sagebrush in its natural environment. Sagebrush uses volatile cues emitted by clipped self or neighbor's branches to increase resistance to herbivory. Widely accepted work states that plants invest more resources in defense under constraining environmental conditions. Here we show that plants that received water during summer and/or volatile cues from clipped neighbor conspecifics accumulated less natural damage than control plants, without showing an interaction between watering and volatile cues. In addition, herbivore damage was negatively correlated with air temperature during summer. We know that activity of herbivorous insects, such as grasshoppers, is strongly affected by air temperature. We suggest that air temperature and precipitation could represent reliable indirect pre-consumptive cues for sagebrush, allowing individuals to adjust resistance to risk of herbivory.

The effect of trophism on metabolom and transcriptional profiles during batch culture growth of microalgae *Chlamidomonas reinhardtii*

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Description of temporal metabolic rearrangements in photosynthetic cells triggered by the trophic conditions is one of the fundamental problem of modern plant biology. The aim of this study is to compare temporal dynamics of metabolomic and transcriptomic profiles of *C. reinhardtii*, constantly growing in a batch culture under mixotrophic (TAP) or autotrophic (TP) conditions as well as after contrast shift from one to another trophic status. GC-MS analyzes revealed nearly 300 metabolites, including 100 identified ones. Expression level of 32 genes, involved in the metabolism of acetate, energy and plastic metabolism and transport plastid metabolites, were tested by RT-PCR. Unsupervised (PCA, RF, HCA) and supervised (PLS, OPLS and RF) mathematical methods were applied for data analysis Multivariate analysis clearly demonstrated dynamics of metabolome during culture development. Lipids and sugars demonstrated the highest link with time of the culture development. Hierarchical cluster analysis allowed identification three major groups of metabolites, depending on the temporal dynamics under mixotrophic conditions. First group encompassed compounds which concentration preferably maximized at the stationary phase. Typical metabolites of this group are long chain saturated fatty acids and diacylglycerols. Other two groups included metabolites with maxima at the period of high physiological activity which fell on the exponential phase. These groups contained short and unsaturated fatty acids, large number of sugar and intermediates of energy cycles. Specificity of metabolites of the third cluster was the second maximum at the end of the log-phase and early stationary phase. Dispersion of metabolite concentrations was much stronger affected by current trophic regime then previous

acclimation. Classification revealed the prominent role of fatty acids, especially unsaturated and moderately long as well as sugars in trophic adaptation.
Further transcriptional analysis completed with multivariate statistics showed that the profiles clear clustered according to the age of Chlamidomonas culture. In the case of mixotrophic culture genes tightly related to culture development involved in acetate assimilation (*ACS1,2*, *ACK1*), starch degradation, in the OPPP and the Calvin cycle, glycolysis and gluconeogenesis (*TRK1*, *PCK1*, *TAL2*, *FBA3*, *HXK1*, *TPIC*, *CIS2* etc.). The expression of these genes was maximal during exponential growth, and then decreased. Characteristically, the genes of transporters exporting photoassimilates from chloroplast, demonstrated a high level of expression under autotrophic conditions. Under mixotrophic conditions more active genes was ones encoding transporters associated with triose shunt between the plastid and the cytosol. During the mixotrophy also more actively expressed genes of acetate assimilation, fatty acids synthesis, gluconeogenesis, Rubisco at the same time genes of Calvin cycle and carbohydrate metabolism in general more active in the autotrophic cells. It should be noted that the differences faded away when culture came to the stationary phase. Preceded acclimation played a relatively weak role in determining of the gene expression profile.
After that a correlation analysis of the dynamics of the each gene expression during culture development under different trophic conditions and acclimation was performed. It was found that a half of the studied genes demonstrated a positive correlation under mixotrophic and autotrophic conditions. The differences mainly regarded to the genes induced at the presence of acetate. Acclimation affected the temporal pattern of expression only for a quarter of genes.
Final correlation analysis identified relationship between gene expression and the metabolite profile. Metabolic map built on obtained data clearly showed two clusters: one cluster, generally, consisted of lipids, grouped around the genes encoding enzymes involved in fatty acid biosynthesis, carbohydrate metabolism, plastid triose transporters. The second smaller cluster, predominantly combined carbohydrates, concentrated around the genes of plastid exporters and genes of starch synthesis and degradation. It was shown that the contrast change in trophic status resulted in aggregation of clusters due to the increased correlations, which can be a symptom of adaptation processes occurring in the cell.
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Serotonin and Melatonin: Their Functional Role in Plant Signaling

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Serotonin and melatonin, two major indoleamines in plants, exhibit a crucial role in the plethora of events involved in these biological processes. Serotonin and auxin biosynthetic pathway is regulated by tryptophan metabolism in developing seeds, thus affecting serotonin accumulation in seedlings. Abiotic stress acclimatization in seedlings has been observed to be modulated by endogenous and exogenous levels of these indoleamines. We have studied the immunolocalization of SER in different tissues of *C. canephora* has revealed that it is localized in vascular tissues of stems, roots, and somatic embryos, as well as in endocarps (husks) of immature fruits. Our results indicate that exogenously supplied serotonin/melatonin induce somatic embryogenesis in cultured tissues of *Coffea*. Studied the interplay of indoleamine neurohormones viz. serotonin, melatonin and calcium channels on shoot organogenesis in *Mimosa pudica* L. Moreover, exogenously fed serotonin/melatonin induce anthocyanin production in cultured tissues of *Daucus carota*. This presentation highlights the detail the role of serotonin and melatonin in plant growth development and complex functions of melatonin and serotonin in the environmental adaptation in plants.

Root signals under hypoxia conditions alter non-functional stomata to functional stomata in low VPD-exposed plants

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Function of stomata is vital for plants to have a balance between absortion of CO_2 for photosynthesis and preventing of wilting because of excessive water loss. By using chlorophyll fluorescence imaging under non-photorespiratory condition (which is indicative of stomatal opening and closure), we showed that there is a difference in the stomatal response to stimuli that promote closure of the stomata between fava bean plants grown at moderate (1.17 kPa) or exposed for 4 days to low (0.23 kPa) vapour pressure deficits (VPDs). Contrary to functional stomata in moderate VPD-grown leaves, exposure of fava bean to four days low VPD made stomata incapable of adequate response to exogenous application of abscisic acid (ABA). Applying hypoxia conditions in the root medium during exposure of the plants to low VPD maintained the functionality of the stomata in response to ABA. The concentrations of ABA and ABA-GE (in the leaf) and hydrogen peroxide (H_2O_2) and nitric oxide (in the stomatal guard cells) were considerably increased after application of hypoxia conditions to the root medium of low VPD-exposed plants. In order to find which signal maintained closing response of stomata, instead of hypoxia, ABA, nitric oxide donor (SNP) and hydrogen peroxide were applied to the root medium and sprayed on the leaves during exposure of plants to low VPD. ABA induced stomatal closure in both root and foliar application, while SNP and hydrogen peroxide treated-plants were less responsive compared to the ABA treatments. The level of ABA in the leaf of both root and foliar ABA applications increased to at least its level in moderate VPD-exposed plants. It can be concluded that high ABA level in the leaf of low VPD-exposed plants, induced the production of nitric oxide and H_2O_2 in the guard cells and maintained the normal functioning of the stomata to stimuli that would normally provoke stomatal closure.

Analysis of interaction between *Arabidopsis thaliana* PCNA and CDKA-1 proteins

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Cyclin-dependent serine/threonine kinases (CDKs) are involved in different signal transduction pathways including stress response. The sequencing of the *Arabidopsis thaliana* genome revealed the presence of genes coding for seven types of CDKs called, CDKA, CDKB, CDKC, CDKD, CDKE, CDKF and CDKG. The activation of CDKs requires the phosphorylation of a conserved threonine in the T-loop region by CDK-activating kinases (CAKs). In addition, the biological activity of CDKs is also dependent on the binding of appropriate cyclins. The aim of this study was the analysis of interaction between Arabidopsis PCNA and CDKA-1 proteins. Using the yeast two hybrid assay and bimolecular fluorescence complementation assay we show that Arabidopsis CDKA-1 can form a complex with PCNA1 and PCNA2 proteins.

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The participation of the Ca^{2+} signal system and biologically active oligosaccharide in the regulation of IAA-induced adventitious roots formation.

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Despite the fact that the many components of the auxin signal transduction have been identified, the molecular mechanism and intermediates of signal transduction the auxin-promoted roots formation remains not clear yet. A wide range of inhibitors has been investigated on the formation of adventitious roots on the buckwheat's hypocotyls explants. The number of adventitious roots was reduced by the action of L-type Ca^{2+}channel blockers (verapamil, diltiazem) and compounds affecting the release of intracellular Ca^{2+} from the vacuole (ruthenium red, neomycin). The application of calmodulin antagonist (chlorpromazine) also decreased the roots number per explant. In contrast, another calmodulin antagonist (fluphenazine) increased the roots number. We believe the fluphenazine effect in this case is directed at the increase of the endogenous cGMP level through the inhibition of the phosphodiesterase (PDE) activity because the Ca^{2+}-calmodulin complex was identified as effector of the plant phosphodiesterase. Taken together, our results present an evidence that the signaling cascade with involvement of cytosolic Ca^{2+} coming both from the outside and intracellular stores causes the formation of adventitious roots. However, all these signaling cascades run downwards the IAA flow. But still there is no information about the signaling molecules functioning before auxin though they are of considerable interest. Such molecules may be oligosaccharide fragments of cell wall polysaccharides that exhibit biological activity. We got oligosaccharin which stimulates IAA-induced root formation especially when added for the short time (1-2 hours) before a hormone. The oligosaccharin does not affect the rooting if this process was already initiated by IAA. The experiments with different combinations of IAA, oligosaccharin and Ca^{2+}- channel blocker (diltiazem) have shown that inhibitor eliminates the effect of IAA but not of oligosaccharin. It can be assumed that the voltage-dependent calcium channels of plasma membrane are involved in the process of the IAA-induced adventitious roots formation on buckwheat explants. The identified by us fraction of oligosaccharin acts at the early stages of root formation before the hormone and it effect is mediated not through the opening of voltage-dependent calcium channels but through any other way. The role of different signaling pathways in the adventitious root formation is discussed.
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Plant Behavior, Flipped

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The subject of “plant behavior” is attractive to biology students at all levels. It can be satisfyingly challenging to experienced undergraduate, and graduate students in biology wishing to test their knowledge of genetic mechanisms, cells, development, signaling, communication, ecology and evolution. For the same reasons that “Plant Neurobiology” provoked controversy, learning about plant behavior provokes students to confront biases and misconceptions, and construct a more informed idea of how plants work.
For the past five years, the UW Biology Department has offered Biology 422, The Physiological Basis for Behavior in Plants. Following a “flipped” classroom structure, students are given information to read and learn prior to coming to class, and asked to utilize their knowledge in class through various activities. Most students in this class have minimal plant biology background; they are majoring in physiology with the goal of entering the medical profession. As there is not sufficient time to deliver a classical knowledge set for plant biology and physiology, students are asked, in class, to construct the knowledge they need to answer questions about plant behavior.
In 2016, students read Brilliant Green (Mancuso and Viola 2015), and Plant Behavior and Intelligence (Trewavas 2014). In past years, students read The Restless Plant (Koller and Van Volkenburgh 2010) and What a Plant Knows (Chamowitz 2014). Prior to attending class, students are asked to read selected publications, view several videos, view and understand information provided in power point presentations about relevant physiological topics, and hand in answers to homework questions designed to guide their reading. This year, the students have two projects making up the bulk of their work: (1)

students are designing an experiment to test whether roots display collective behavior (Sasaki and Pratt 2011, Sasaki et al. 2013), and (2) they are contributing a 4-paragraph, referenced article to Wikipedia on a topic related to plant behavior.

The flipped classroom structure augments the attractiveness of plant behavior as a topic. Students are guided in their reading outside of class, and stimulated to learn as a group in class. Provided behavior-based knowledge in plant physiology, students learn and internalize much about how plants (and other biological organisms) sense, learn, remember, and act.

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SESSION 7

Phytohormone Signalling

The effect of different concentrations of hypericin hormone stimulates the production of the plant Hypericum

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Naphtodianthrone is a light-sensitive hypericin photodynamic activaity. Hypericum extracts and compounds from the plant is useful in the treatment of many diseases. The research was conducted in the Laboratory of Biotechnology and Plant Breeding of Islamic Azad University. In this study, the effect of different concentrations of hormones 2.4-D+BAP and NAA+KIN in basal medium (MS) to stimulate the production of hypericin was investigated. Tiny samples of the leaves and stems of sterile plants cultivated in basal medium (MS) has been prepared and the MS medium containing 2.4-D+BAP and NAA+KIN hormone concentrations (control, 0/5 and 1 mg l) were transported at a temperature of 21 ± 3 °C in 16 hours of light and 8 hours of darkness and light conditions were maintained. Methanol extracts of this plant by HPLC analyzes to examine changes in hypericin. The results showed that hypericin range of chromatogram standard range of hypericin in 05:38 minutes also show the whole standard of hypericin. So the percentage of hypericin in the control sample of 1% is at 06:02. Different concentrations hormones 2.4-D+BAP and NAA+KIN reflects the impact on stimulating the production of hypericin in the plant is not actually had any effect on stimulating the production of hypericin in vitro. Therefore it is recommended that hormones or other growth regulators can be used to stimulate the production of hypericin.

Importance of apoplast pathway for the uptake and distribution of zeatin and its riboside in wheat plants

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Free bases of cytokinins and their ribosides are considered as mobile forms of these hormones capable of passing across cell membranes. Although ribosides are characterized as the transport forms of cytokinins, free bases are also present in xylem and phloem. Still not much is known about the pathways of these hormones in plants. Thus importance of transport with transpiration stream through apoplast for either of these cytokinin forms is unclear. To address this question we studied accumulation and distribution of cytokinins in wheat plants supplied with zeatin or its riboside added to the nutrient medium under conditions of normal and reduced transpiration. Transpiration was decreased by placing wheat shoots into polyethylene bags, where air became water saturated. Since under these conditions transpiration was only 5 % of the control plants, the plants are named below as “nontranspiring”.
Under transpiring conditions, treatment of plants with either zeatin or its riboside increased cytokinin content in plants as detected 6 hours after the start of the treatment. Greater proportion of cytokinins accumulated in the roots, the effect being less pronounced in case of plants treated with zeatin riboside. Reduced transpiration dramatically decreased the level of cytokinin accumulation in plants treated with zeatin riboside, while the level of accumulation remained almost similar under nontranspiring and transpiring conditions in the case of zeatin-treated plants. Since transpiration maintains the flow through apoplast, the results suggest importance of apoplast pathway for the uptake of zeatin riboside, but not its free base.
There was also a striking difference between zeatin- and zeatin riboside-treated plants in the level of cytokinin accumulation under transpiring conditions that was much higher in case of plants treated with the free cytokinin base. The rate of uptake of exogenous cytokinins measured as the decline in hormone concentration in the nutrient medium was similar in plants treated with either zeatin or its

riboside. Consequently the lower level of cytokinin accumulation in the plants treated with zeatin riboside was likely to be due to its faster decay in the plants. However measuring activity of cytokinin oxidase showed that in the roots enzyme activity was not influenced by hormone treatment, while in the shoots it was increased to the same extent by both cytokinins. Since activity of cytokinin oxidase is likely to be higher in apolast, lower level of cytokinin accumulation in plants treated with zeatin riboside may to be due to is presence in apoplast, where it is more available to the action of cytokinin oxidase.
Immunohystochemical analysis of cytokinins, showed greatly increased immunostaining for cytokinins of the root cells of the zeatin-treated plants, while increment of immunostaining was much smaller in the case of plants supplied with zeatin riboside. The results suggest that uptake of zeatin by root cells prevented their decay by cytokinin oxidase located in the apoplast. Treatment of the plants with protonophore *m*-chlorophenylhydrazone (CCCP) known to inhibit active zeatin uptake by destroying proton gradients resulted in a sharp decline in root cell immunostaining for cytokinins. The effect of CCCP was much weaker in case of plants treated with zeatin riboside. The results suggest great dependence of zeatin uptake by cells on creation of proton gradient acting as a source of energy. Uptake of zeatin riboside by cells was smaller than that of free zeatin and less dependent on secondary active transport. Thus importance of apoplast pathway for the transport of zeatin riboside is supported by several lines of evidence: (1) great dependence of the uptake of this cytokinin form on transpiration, (2) greater decay presumably catalyzed by cytokinin oxidase located in the apolast (3) lower level of cytokinin accumulation in the root cells of plants treated with zeatin riboside as compared to those treated with zeatin.(The work was granted by Russian Foundation for Basic Research N 15-04-04750).

Dynamics of lipoxygenase cascade gene expression in plants of different taxa in various conditions

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Lipoxygenase cascade is the source of the signal compounds, namely oxylipins, playing an important role in the regulation of plant growth and development, cell signaling and protection. Mechanisms of defense may be constitutive and/or inducible and include physiological barriers and production of toxic compounds. Oxylipins play a significant role in plant protection, functioning as signaling molecules and/or protective compounds such as healing and antimicrobial agents. Usually oxylipins involved in defense mechanisms do not synthesized in advance; their synthesis occur de novo in response to mechanical damage, infection or other stress factors. The main enzymes responsible for the plant oxylipins biosynthesis are cytochromes P450 of the CYP74 family: allene oxide synthases (AOSs), hydroperoxide lyases (HPLs), divinyl ether synthases (DESs), and epoxyalcohol synthases (EASs). In this work we studied dynamics of lipoxygenase cascade gene expression under stress factors (biotic and abiotic). The main objects were maize (Zea mays L.), soybean (Glycine max L.), flax (Linum usitatissimum L.) and green moss (Physcomitrella patens). Selection of plant objects was based on interest to study changes in the functioning of lipoxygenase cascade in plants belonging to different taxa: monocots (maize), dicots (flax and soybean) and mosses (P. patens). Lipoxygenase cascade of all objects is different. In flax it is supplemented with DESs, in soybean – with EASs. At the same time, the lipoxygenase cascade of P. patens is complicated by the additional substrate – arachidonic acid (C20). In the flowering plants substrates for lipoxygenases are linoleic and acid α-linolenic (C18). The results indicate that different branches of the lipoxygenase cascade under abiotic stress factors act differently in each plant species.
To identify possible additional inducers of gene expression, we performed an analysis of the promoter regions of the CYP74 genes and identified a large number of sequences specific for genes whose expression is dependent on light. Therefore, changing light conditions has been used as the stress factor. We demonstrated changes in gene expression under changes of light conditions. Additionally, we analyzed change of gene expression under infection with enterobacteria. The biological properties of the individual reaction products were also analyzed. The results of this work indicate that the lipoxygenase cascade is involved in the formation of cell response to infection by pathogenic enterobacteria.
For a long time it was believed that the CYP74 enzymes exist solely in flowering plants. However, our findings indicate a much more ancient origin of this family, which is confirmed by plastid localization of the majority of the CYP74 enzymes, the structure and catalytic action of these enzymes compared to other cytochromes P450, as well as data of phylogenetic studies. Additional evidence is the fact that representatives of the CYP74 clan have been revealed in a wide variety of organisms, including animals, plants, fungi, proteobacteria and brown algae. We have approximately measured the time of occurrence of these enzymes in the evolutionary history and collected evidences to support this assumption. Based

on all these data, including the evolution of enzymes within the CYP74 family, we hypothesized the roles of individual CYP74 enzymes in plant life during evolution.
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Roles of CK2 in Auxin Response, F-Actin Organization and Root Phototropism

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Protein kinase CK2 is a well-conserved pleiotropic Ser/Thr kinase. It is a tetrameric protein composed of two alpha (catalytic) and two beta (regulatory) subunits. In plants, CK2 is involved in the regulation of several important pathways, including light signaling and stress-responsive pathways. In order to study the functional implications of CK2 in plants, we have worked with a CK2 dominant negative mutant (overexpressing an inactive catalytic inactive CK2α subunit), previously generated in our laboratory (Moreno-Romero et al., Plant J 2008, 55, 118).
As an alternative strategy of inhibition of enzymatic activity, we have used 4,5,6,7 tetrabromobenzotriazol (TBB), a specific inhibitor of CK2. The loss of CK2 activity revealed interesting phenotypical changes in auxin dependent processes, many of them related with defects in the polar auxin transport (PAT). PAT controls important growth and developmental processes and it is also implicated in responses to directional light sources. We have investigated the gravitropic and phototropic responses in CK2mut plants, and the distribution of auxin during lateral blue light inductions. To do this, we have used the yellow fluorescent protein VENUS fused to the Aux/IAA auxin-interaction domain (DII) and expressed under a constitutive promoter. Auxin has important roles in the reorganization of the actin cytoskeleton. We have investigated F-actin architecture under conditions of CK2 activity depletion, using the GFP-FABD2 F-actin line (a stably transformed line with a fusion construct in which N-terminal GFP is fused to C-terminal half of AtFimA, which includes the second actin-binding domain and the C-terminal end *of A. thaliana* fimbrin 1). We have also studied the effect of phosphatidic acid (PA) on GFP-FABD2 plants after the inhibition of CK2 by TBB. We have found dramatic F-actin depolymerization, due to the loss of CK2 activity, is recovered after PA treatment. PA binds to the capping protein and promotes actin polymerization.
Finally, we have studied also the stability of AUX/IAA proteins, which are repressors of auxin signaling pathways, modulating auxin responses. We have used transgenic plants in which a construct with the AX3 domain fused to GUS (beta-glucuronidase) was placed under the control of the soybean heat-shock promoter HS. Our results show that the inhibition of CK2 activities increases the stability of the AUX/IAA proteins, blocking the activation of auxin-regulated gene transcription.

The effect of brassinosteroids on growth and development of *Phalenopsis* protocorm-like bodies

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Introduction. The content of certain phytohormones and their concentrations in a medium is the determining factor for controlling growth and differentiation of plant cultured *in vitro*. The most commonly used hormones are auxins and cytokinins. Recent studies showed that brassinosteroids (BRs) have a strong modifying effect on growth, development, sex determination and reproduction in higher plants. However these hormones are not studied for their action on growth of plant *in vitro* cultures. Moreover their effects are not investigated in such an important plant as orchids.
The aim of this work was to determine the effect of six different BRs, belonging to two main BR classes, on growth rate and development of *Phalaenopsis* · hybridum Blume protocorm-like bodies. 10^{-10}-10^{-6} M brassinolide (BL), castasterone (CS), epicastasterone (EC), homocastasterone (GC), epibrassinolide (EB) and homobrassinolide (GB) were tested. Culture of protocorms was generated from seeds of *Phalaenopsis* · hybridum Blume. Protocorm-like bodies were isolated from the primary culture and transferred to media containing various levels of BRs. Weigh and length of the protocorm-like bodies were measured after 100 days of cultivation on BR-containing media. Our data demonstrated that all

BRs significantly stimulated orchid growth *in vitro*. The greatest effect on length was caused by CS while maximal increase of weight was induced by BL and EB. Orchid microclones, grown in the presence of 10^{-6} M CS, had twice bigger length that control plants. Weight gain also increased 2 and 3.5 times when plants were cultivated on media containing 10^{-8} M and 10^{-6} M BL, respectively. GB and GC caused smallest effects on growth among all tested BRs. We also compared the BR effects with classical auxins, such as indol-3-acetic acid, indole-3-butyric acid and 2,4-dichlorophenoxyacetic acid. We have found that auxins were less effective than BRs.

Conclusion. We have demonstrated for the first time that BRs stimulate growth of *Phalaenopsis* · hybridum Blume protocorm-like bodies and that this stimulation exceed effect of auxins.

Early cellular events and auxin response during lateral root initiation in the primary root meristem of squash (*Cucurbita pepo*)

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Formation of lateral roots takes place directly in the parental root meristem in several Eudicots plant families such as Cucurbitaceae and Polygonaceae. However, so far there are no reliable data on the earliest events in this type of lateral root initiation neither on the molecular nor on the physiological level. Here, we present data on the earliest events in lateral root initiation in squash (*Cucurbita pepo* L.) and show how organogenesis takes place among proliferating cells.

In order to detect the cellular response to auxin, a new DR5::GFP-GUS-NLS vector was constructed carrying reporter an NLS (Nuclear Localization Signal)-GFP fusion. Another improved DR5::tdTomato-H2B vector containing a *pUBQ10::H2B-Venus* screening cassette was constructed as well. As the tdTomato protein belongs to the brightest fluorescent proteins, the *tdTomato-H2B* fusion allowed detection of even relatively weak responses to auxin in inner layers of the root, and thanks to the use of H2B instead of NLS, even in dividing cells. The *pUBQ10::H2B-Venus* cassette was used to label nuclei and also permitted effective identification of transgenic roots. Composite squash plants harboring these inserts were obtained using a technique developed previously (Ilina et al. 2012 *Annals of Botany*). The nuclear localization of reporter protein allowed a more sensitive detection of the auxin response than cytosolic GFP or GUS. Localization of the first anatomical events in lateral root initiation was carried out on longitudinal root sections (60 μm) by 3D reconstruction of series of optical sections using a confocal laser scanning microscope LSM780 (Zeiss, Germany). Localization of the cellular response to auxin was detected based on the accumulation of GFP or tdTomato in the nuclei of cells.

The cellular response to auxin took place in sister cell pairs (founder cells) directly before the first anticlinal division that initiated the formation of a lateral root primordium in our model system: divisions in the inner and outer layers of pericycle and endodermis. These events took place at a distance 250-300 μm from initial cells. Altogether, cell pairs in two to three longitudinal files of both pericycle layers and one file of the endodermis were involved in lateral root initiation. Later the number of endodermal cell files increased to up to three, and also cells from the inner cortex of the parental root became involved in the formation of the primordium. Remarkably, during the next stages of primordium development only part of proliferating cells maintained the response to auxin. Cells on the periphery of the primordium quickly lost the auxin response. 3D-reconstructions of the earliest events in lateral root initiation and later stages of lateral root primordium development will be presented.

Additional experiments were performed to identify the triggers of lateral root initiation in Cucurbits. Squash seedlings were cultivated in hydroponic culture for four days in the presence of one of growth regulators. It was shown that different forms of exogenous auxins, active auxin transport inhibitors and regulators of ethylene synthesis and signaling had no influence on the number of lateral roots, and thus on lateral root initiation, in our model system.

Our results show that the local increase of the cellular response to auxin in Cucurbits is not required to determine the lateral root initiation site and to maintain cell divisions in the primordium but for determining the direction of the primordium growth and thus, the structure of the lateral root.

Furthermore, data on promoter activity of several meristem-specific genes in squash root tips will be shown and discussed with respect to the influence of these genes on lateral root initiation. Special attention will be paid to WOX5 (meristem-specific transcription factor), SCR (genetic marker of the endodermis), CR4 (receptor-like kinase, regulator of cell proliferation) and ALF4 (Aberrant Lateral Root Formation 4, regulator of the ability to resume the cell proliferation above the elongation zone by lateral root initiation).

The design and construction of vectors was financially supported by the Russian Science Foundation (16-16-00089). This work was additional supported by the Russian Foundation for Basic Research (14-04-01413-a).

Root developmental plasticity: to maintain the root apical meristem or become determinate?

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Developmental plasticity is an important component of plant adaptation. During root system formation at least two developmental programs evolved, one is related to the root apical meristem (RAM) maintenance and another to the RAM exhaustion. How the decision is taken to maintain the RAM or transit to the RAM exhaustion is mainly unknown. The RAM maintenance permits root indeterminate growth and when the RAM is exhausted the root becomes determinate. We are pursuing the goal of understanding the root developmental plasticity from evolutionary and ontogenetic perspectives. We implement two approaches: (1) RNA-seq of the primary root apices of Cactaceae species that have constitutive determinate growth and (2) *Arabidopsis thaliana* mutant screening and identification of genes responsible for mutations that alter root growth.

We found that in cactus species with determinate growth the RAM persistence and its exhaustion are related to the transient establishment of the quiescent center (QC) and limited activity of initial (stem) cells. Currently we analyze the gene expression profiles in cactus roots at stages when the RAM is present and when it is exhausted. Importantly, when the determinacy program is on, differentiation-related genes are more abundant. This approach allowed the identification of new candidate genes that may be involved in the RAM maintenance or exhaustion.

We analyzed loss-of-function mutants affected in root growth. Some of these mutants have been previously described (A*rabidopsis homolog of trithorax1, atx1,* Alvarez-Venegas *et al.,* 2003; *Curr Biol* 13: 627) and some were isolated in our laboratory (*moots koom* ['short root' in Mayan]*1*, *mko1,* and *mko2).* Analyzing *atx1* mutant, we found that *ATX1*, coding for an H3K4-histone methyltransferase, is required for cell production, patterning, and morphogenesis in root development. As a consequence, growth of *atx1-1* root was significantly slower than in wild type. The expression site of the QC-specific markers, *WOX5* and *QC46,* was expanded in the *atx1-1* RAM independently of auxin-response gradients. Interestingly, the root indeterminacy was maintained, suggesting *ATX1* participation in the RAM maintenance only.

Analyzing *mko1* and *mko2*, both of which showed determinate root growth we found that the mutant phenotype is caused by a point mutation in *THREONINE SYNTHASE1* and *FOLYLPOLYGLUTAMATE SYNTHETASE1,* respectively. When *mko1* and *mko2* seedlings grew in presence of threonine or 5-formyl-tetrahydrofolate, respectively, root growth determinacy was reversed to indeterminacy. Both genes are required for the post-germination maintenance of the RAM. The QC identity markers in *mko1*, *pWOX5::GFP* and *pSCR::GFP,* were correctly expressed during the early stages of the RAM exhaustion. Alterations in folate metabolism in *mko2* caused the loss of stem cell activity and induced the QC cell division. Our data indicate that the folate-dependent pathway of the indeterminacy-to-determinacy switch operates independently of auxin gradients and known regulatory modules that participate in the RAM maintenance (WOX5, PLETHORA, and SCR). We conclude that a developmental program for the RAM maintenance is distinct from that of the maintenance of root indeterminacy. When the former program is compromised, a smaller meristem is preserved, whereas when the latter program does not operate, the whole meristem becomes consumed. Therefore, RAM maintenance and the maintenance of indeterminacy may represent different developmental pathways in the regulation of the RAM behavior.

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Azelaic acid effect on the pea root proteome

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It is known that pathogens are able to induce immunity not only at the site of infection (local immunity), but also in distal tissues (system immunity or systemic acquired resistance (SAR)). The letter is induced by a number of transported signals, including the newly revealed azelaic acid (AzA). AzA is nine carbon dicarboxylic acid generating from plastid galactolipid oxidation during infection. It has been shown that AzA implicated in the SAR-associated priming of salicylic acid (SA) accumulation in Arabidopsis. But nothing is known about the transport of AzA to the plant roots and its effect on plant root proteomes.
Earlier in the experiments studying the effect of SA on the pea roots oxylipins spectra we found the increased content of AzA. Exogenously applied AzA caused reduction of plant growth; length of roots and shots was shortened in AzA treated plants compared to control. We have revealed that the content of soluble protein is not significantly changed under the action of AzA on pea plants. For the first time we conducted the proteomic analysis of the AzA effect on pea root proteomes and revealed a number of AzA-dependent proteins. Under the action of AzA the content of 27 soluble proteins from about 600 detected on 2DE gels was changed. The content of ABA-responsive protein and isoflavone reductase participating in the synthesis of antipathogenic phytoalexins synthesis was increased. NADPH oxidoreductase, aconitate hydratase were increased under the action of AzA. The content of proteins participating mainly in the protein metabolism (protein disulfide isomerase, disulfide-isomerase, 14-3-3 proteins and HSP70) and in the metabolism of nucleic acids (ribonucleoprotein, DEAD box RNA helicase) was decreased. The content of ATP synthase subunit delta glucose-6-phosphate isomerase, V-type proton ATPase subunit E, L-ascorbate peroxidase, two forms of actin were decreased. Decrease of the actin content may be due to the shortening of the pea plant roots under the action of AzA.
If we have in mind that AzA is implicated in the priming of SA in Arabidopsis, we can suppose that SA- and AzA dependent proteins could be similar. But AzA – dependent and SA-dependent proteins in pea roots in general were different. We can suppose that AzA itself is able to affect the plant proteomes. It is necessary to carry out further studies to obtain more information about the role of AzA in the pea roots defense reactions.

The lipoxygenase signaling system of several species of brown algae

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Plants lack an immune system in the sense that it exists in animals, but they possess mechanisms that recognize potential pathogens and initiate defense responses. It has become evident that various types of oxygenated fatty acids, collectively termed «oxylipins», are involved in responses to biotic and abiotic stress factors. These compounds include, for instance, the eicosanoids produced from arachidonate in animals, which have so many varied functions, especially in the inflammatory processes.
Cytochromes P450 – haem-thiolate enzymes common for all eukaryotic organisms. This superfamily consists of hundreds families and several clans. The most unusual enzymes among cytochromes P450 belong to the CYP74 clan. Almost all cytochromes P450 are monooxygenases and they metabolize two substrates: oxidized compound and molecular oxygen. Unlike the monooxygenases, the CYP74 enzymes have an atypical reaction mechanism that requires neither oxygen nor redox partners. The CYP74 clan includes hydroperoxide lyases (HPLs), allene oxide synthases (AOSs), divinyl ether synthases (DESs) and the most poorly studied epoxyalcohol synthases (EASs). Despite differences in product specificities, they all metabolize 9- and/or 13-hydroperoxides as substrates, which are produced by the oxygenation of polyunsaturated fatty acids by the action of lipoxygenases (LOX).
The CYP74 clan members have been found in a wide variety of organisms including animals, plants, bacteria, but not algae. Despite this fact oxylipins were detected in different algae species. We analyzed patterns of endogenous oxylipins in several species of brown algae: *Undaria pinnatifida, Sargassum miyabei, Sargassum pallidum* and *Saccharina cichorioides*. Different isomers of epoxyalcohols, ketols, hydroxy and trihydroxy acids were detected. The structures of all compounds have been resolved using GC-MS, NMR and UV spectroscopy.

Analysis of genome data of brown alga *Ectocarpus siliculosus* with open reading frames revealed 12 sequences which have homology with cytochromes P450 of higher plants. One of them has similarity with the CYP74 enzymes. We obtained corresponding recombinant protein using *E. coli* expression system. Affinity-purified protein EsCYP74 was incubated with model substrates – 9- and 13-hydroperoxides of linoleic acid. Incubation resulted in formation of a number of epoxyalcohols, di- and trihydroxy acids. Thus, the described enzyme EsCYP74 is the first member of the CYP74 clan detected in algae, and it belongs to one of the most poorly studied groups of the CYP74 enzymes – epoxyalcohol synthase. Detection of the CYP74 enzyme in brown alga complements the common scheme of the CYP74 catalysis and confirms our hypothesis of the CYP74 clan origin and evolutional history of P450 superfamily.

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Concentration and distribution of cytokinins in P-starved barley plants and its dependence on secondary active transport

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Plants are capable of maintaining their root growth despite shortage of essential ions, this response enabling efficient acquisition of mineral nutrients. Allocation of carbon to root growth is brought about by inhibition of shoot growth. This response usually takes place before a decline in the internal concentration of ions in the plants brought about by a drop in the concentration of nutrients in the medium. It is believed the inhibition shoot growth is due to some early signals of nutrient shortage perceived by roots are transferred to the shoots. The decline in cytokinin delivery from the nutrient-starved roots to shoots is the likely candidates for the role of such a signal. These hormones are known to be necessary for the shoot growth, while their concentration in plants is frequently decreased by the shortage of essential nutrients. Thus, the decline in shoot growth under nutrient deficiency is believed to be due to a decline in cytokinin delivery from the roots. However, it remains not clear enough how a fast decline in cytokinin export from roots may be brought about. Dilution of mineral nutrients was shown to activate cytokinin oxidase thereby resulting in a decline in cytokinin content in plants (Vysotskaya et al., 2009). It was of interest to find out, if this or any other mechanism operates in case of P-starved plants.

Experiments were carried out on the barley plants (*Hordeum vulgare* L.) cv. Prairie. Seeds were allowed to germinate for 3 days floating in sealed and tied together glass tubes at 24 °C and after that they were transferred to modified 0.1 strength Hoagland–Arnon nutrient medium, where KH_2PO_4 was either omitted (P^-) or substituted with NaH_2PO_4 (P^+), and were grown at a 14-h photoperiod and an irradiance of 400 μmol m^{-2} s^{-1}. Protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was added to half of both P^- and P^+ plants to yield 10 μM concentration. One day after the start of P-starvation, plant samples were assayed for hormone and, on the 5th day, shoot and root mass of the rest of plants was measured.

Five days after P withdrawal from the nutrient medium, shoot mass of barley plants was lighter than of those supplied with P, while root mass was similar in both P^+ and P^- plants. This resulted in greater root/shoot mass ratio in plants deprived of phosphorus (root/shoot mass ratio being 0.46 and 0.54 in P^+ and P^- plants, correspondingly).

Withdrawal of phosphates from the nutrient medium did not affect significantly the sum of cytokinins (zeatin, its riboside and nucleotide) on the whole plant level (17 pmol and 13 pmol in P^+ and P^- plants, correspondingly). Effects on organ level were much greater and P-starvation induced changes in shoot cytokinins reached 4.5 decline when calculated per whole shoot and 3.6 decline when calculated per g of shoot fresh weight. In roots, opposite response was observed, cytokinin content and concentration being about 3 times higher in P^- plants than in P^+ plants.

Activation of cytokinin decay is not likely to function in P^- barley plants, since whole plant cytokinin content remained unchanged in the plants. Instead, accumulation of cytokinins in roots of P^- plants suggested that the detected drop in shoot cytokinins may be attributed to inhibition of transport of this hormone from the roots. Importance of active cytokinin uptake by roots cells to prevent their loading into xylem was recently reported (Kudoyarova et al. 2014) and this mechanism may function in P-starved barley plants. To check this assumption we treated plants with protonophore CCCP. Inhibition of secondary active transport by CCCP resulted in a decline in cytokinin accumulation in the roots of P-stared plants and concomitant increase in the shoot cytokinin content. This may serve as evidence that the decline in shoot cytokinin content and accumulation of the hormones in roots of P-starved plants was due to active cytokinin uptake by root cells preventing their flow to the shoots. In this way

cytokinins may be stored in the roots until the changes in environment may demand restoration of cytokinin delivery to the shoots.
Thus, the results we got reveal a novel mechanism controlling cytokinin distant signaling in P-starved plants. This mechanism involves active cytokinin uptake by root cells as a mean to decrease the transportation of there hormones from the roots to the shoots.
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Cytokinins determine arabidopsis root meristem size and root growth rate by controlling cell proliferation rather than cell differantation

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The transition of meristematic cells to elongation is needed for maintenance of the root growth rate since the duration of the root cell elongation is very short. The rate of cell transition to elongation (differentiation) is regulated by two groups of independent processes. The first ones regulate the life-span of cells in meristem – the duration of the period when cells located at various distances from the root tip reach the basal boundary of meristem and begin to elongate. The seconds one regulate the rate of the cell proliferation during the life-span of cells in meristem. Trans-zeatin slows down the root growth rate and the rate of the transition of meristematic cells to elongation as a result of prolongation of mitotic cycles. The life-span of cells in meristem does not change. The number of meristematic cells in one file decreases but it is the result of the inhibition of cell proliferation but not the acceleration of the cell transition to elongation (differentiation). The roots of triple mutants *ipt3ipt5ipt7* in which cytokinin synthesis is slowed down grow at higher rate than control roots. The number of meristematic cells in one file in these roots is greater than in roots of wild type. It is the result of the higher rate of cell transition to elongation and the acceleration of cell proliferation. The life-span of cells in meristem is shorter than in control roots. Analogical results were obtained for roots of some cytokinin signaling mutants. Thus, slowing root growth, when treated with cytokinins, or acceleration growth with reduction of the concentration of endogenous cytokinin or weakening of their signaling are associated with changes in the duration of mitotic cycle.
Alyhough, there is a view that cytokinines regulate the cell transition to differentiation, and thereby changing the rate of root growth and meristem size, our date rather showed that cytokinis affects the cell cycle duration.
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Photoreceptors and phytohormones in the periodic light signal transduction in *Arabidopsis thaliana*

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Plant development is the result of an endogenous morphogenetic program that integrates environmental signals. A major factor in seasonal control of flowering time and photomorphogenesis is the photoperiod. The circadian clock is a set of genes that integrates environmental factors into an internal pacing system, which plays a major role in processes of growth, flowering and other. The circadian clock modulates rhythmic plant growth including the elongation of hypocotyls. Circadian rhythms are endogenous, they are able to adjust the fluctuations of physical and biochemical processes in response to local environmental type cues of daylight. As you know phytochrome (P) are the material bases for light-dependent biological clock. The accumulation of P_R and P_{FR} forms in cells is known to perform many physiological activities within the cell(s). The *cryptochromes* (*Cry*) are circadian clock genes that encode a major component of the circadian clock negative feedback loop. Phytohormones, auxins in particular, play an important role in plant development and productivity. Currently the role of melatonin in circadian rhythms of plants is not studied. In this regard, the aim of the work was to study the role of sensory photoreceptors and phytohormone IAA and melatonin in the regulation of hypocotyl *Arabidopsis* growth on a blue and red light of different photoperiod.
The object of the study was 5-day-old seedlings of long-day plant *Arabidopsis thaliana* (L.) Heynh. ecotype Landsberg *erecta* (L*er*) and its *hy4* and *hy3* mutants, defective, respectively, on photoreceptor blue light / UV-A cry1 and red light/ far red light phyB. Seedlings were grown under blue (BL) and red

(RL) light (125 μmol photons / (m^2s), photoperiod: short day - 8 h - SD, a long day -16 h - DD). Culturing was carried out on sterile 50% MS agar medium supplement with DMSO (control), 0.1 pM melatonin (Mel) or / and 10 μM 1-N-naphthylphthalamic acid (NPA) (experience).
The study showed that L*er* hypocotyl grown under BL was shorter hypocotyl grown under RL. In the absence of cry1 *hy4* hypocotyl lengthened under DD BL, whereas in the absence of phyB *hy3* hypocotyl shortened under DD BL, but lengthened under SD RL and DD RL. These data show the importance of the specific photoreceptors for normal photomorphogenesis under conditions of short and long day. Mel inhibited the hypocotyl elongation of the *hy3* mutant under SD RL and DD RL and lengthened it to under DD BL that restored the hypocotyl phenotype of wild type. During seeding growth, the phytochrome phyB played an important role in the regulation of hypocotyl elongation. Since the IAA regulates the hypocotyl growth, it can be assumed that the change in its growth related to the change in the level of endogenous IAA. It can be assumed that the hypocotyl growth change has been related of the change of the IAA level because IAA regulates the cell elongation. Application NPA did not change the hypocotyl length of wild type L*er* SD BL and SD BL, but reduced its *hy4* and *hy3* mutants. Treatment NPA + melatonin inhibited the elongation of seedlings hypocotyls under different photoperiods. At the same time Mel enhanced the inhibitory effect of NPA on the hypocotyls growth of wild type under DD BL, but he cleaned the inhibitory effect of NPA on the *hy3* hypocotyl growth under SD BL. Treatment with auxin efflux inhibitors such as NPA possibly led to changes in PIN polarity that defines IAA level. These results suggest that photoregulation of the IAA and melatonin levels must also be taken into account for a better understanding of the molecular mechanisms underlying circadian rhythms. We propose that the sensor governing circadian growth responses defines a pathway that depends on the functional state of phyB and cry1, and the IAA and melatonin levels.

Ethylene signaling affected by silenced ACC oxidase genes in banana

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Ethylene is a crucial hormone which involved in fruit ripening of banana (*Musa* spp.). To elucidate the signal transduction pathway involved in the ethylene regulation of banana ripening, gene expression patterns that relate to signal transduction and ethylene biosynthesis were performed by real-time reverse transcription-polymerase chain reaction (qRT-PCR) in two distinct ACC oxidase genes *Mh-ACO1* and *Mh-ACO2*-silenced bananas, respectively. For those genes responding to ethylene biosynthesis, the results showed that not only the *Mh-ACO1* gene had a significant silenced effect at stages 4 to 6 of peels from *Mh-ACO1*-silenced transgenic banana but also the *Mh-ACO2* gene was suppressed indicating an interaction between these two ACC oxidase genes exists. In peels of the *Mh-ACO2*-silenced transgenic banana, the best silencing effect on *Mh-ACO2* was shown at the stages 5 and 6 of peels after increased expression at stage 4 with very low expression level of *Mh-ACO1* in all the ripening stages. For genes involved in ethylene signal transduction, ethylene receptor *Mh-ERS1*, negative regulator *Mh-CTR1*, positive regulator *Mh-EIN2*, and transcription factor *Mh-EIL1* all expressed lower in the peels of *Mh-ACO1*-silenced banana than in wild type. Generally, the expression levels of four ethylene signaling genes were similar to wild type except *Mh-ERS1*, *Mh-CTR1*, and *Mh-EIN2* had more expression peaks than wild type at stage 6, stage 4 and 6, and stage 4, respectively, in peels of *Mh-ACO2*-silenced transgenic banana. However, in the pulps of *Mh-ACO1*-silenced banana, the expression levels of four ethylene signaling genes were much lower than wild type. As studied previously, *Mh-CTR1* expression in pulps serves as a trigger to produce large amounts of ethylene in the fruit *via* a cascade of cellular events. The expression peak of *Mh-CTR1* expression in both wild type and the *Mh-ACO2*-silenced transgenic banana were at stage 3 with higher expression in *Mh-ACO2*-silenced transgenic banana. Apparently, lower expression of *Mh-ACO2* gave rise to an increase of *Mh-CTR1* expression in banana fruits. The expression of *Mh-ERS1* reached maximum at stage 4 in pulps of *Mh-ACO2*-silenced transgenic banana but not in wild type. The expression levels of *Mh-EIN2* in peels of wild type and both transgenic bananas were similar except one more peak showed at stage 4 in the *Mh-ACO2*-silenced transgenic banana. Not like wild type, no expression peak of *Mh-EIL1* appeared in *Mh-ACO2*-silenced banana. Based on the qRT-PCR analysis shown here, it is indicated that expression of genes involved in the ethylene signal transduction pathway was strongly influenced by the expression of genes associated with ethylene biosynthesis. Our results offer an opportunity to reveal the molecular mechanism underlying fruit ripening in banana.

Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity

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Two major phytohormones, auxin and cytokinin, contribute together to the control of a multitude of plant developmental processes. Despite their crucial roles, comparison of the exact distribution profiles of different hormones within plant meristems has thus far remained scarce. Due to the fact that secondary development of a tree trunk takes place through the activity of vascular cambium, a wide lateral meristem with an extensive developmental zonation, it provides an optimal system for hormonal and genetic profiling. By taking advantage of this spatial resolution, we show here that the two major plant hormones, cytokinin and auxin, display different, yet partially overlapping, distribution profiles across the cambium. In contrast to auxin, which has its highest concentration in the actively dividing cambial cells, cytokinins peak in the developing phloem tissue of a *Populus trichocarpa* stem. Gene expression patterns of cytokinin biosynthetic and signaling genes coincide with this hormonal gradient. To explore the functional significance of cytokinin signaling for cambial development, we engineered transgenic *Populus tremula* x *tremuloides* trees with an elevated cytokinin biosynthesis level. Confirming that cytokinins function as major regulators of cambial activity, these trees displayed stimulated cambial cell division activity resulting in dramatically increased (up to 70% in volume) production of the lignocellulosic trunk biomass. To connect the increased growth to hormonal status, we analyzed the hormone distribution and genome-wide gene expression profiles in unprecedentedly high resolution across the cambial zone. Interestingly, in addition to showing an elevated cambial cytokinin content and signaling level, also the cambial auxin concentration and auxin responsive gene expression, were increased in the transgenic trees. Our results indicate that cytokinin signaling specifies meristematic activity through a graded distribution that influences the amplitude of the cambial auxin gradient.

Genetic regulation of the initial steps of Cucurbitaceae lateral root initiation: role of the *ABERRANT LATERAL ROOT FORMATION 4* (*ALF4*) gene

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Understanding the physiological and molecular mechanisms underlying lateral root initiation is a current problem of plant development. Our research object is squash (*Cucurbita pepo*) which is characterized by lateral root initiation within the parental root meristem. For this type of root branching, hormonal and genetic interactions that control root branching in the representatives of the cucurbit family are still poorly understood.

The *ABERRANT LATERAL ROOT FORMATION 4* (*ALF4*) gene characterized in *Arabidopsis* encodes a protein unique to plants, presumably of nuclear localization, which has no similarity with any other known protein. *alf4* mutants do not form lateral roots (DiDonato et al., *Plant Journal*, 2004). Their explants are not able to produce calli (Sugimoto et al., *Developmental Cell*, 2010). These data indicate that the *ALF4* plays an important role in lateral root initiation.

Expression of the *ALF4* promoter in transgenic squash roots harbouring a *pCpALF4::Egfp-gusA* construct was studied based on GUS activity in root tissues. Expression patterns of the reporter constructs containing 1298 bp and 2012 bp, respectively, of the squash *ALF4* promoter were different. Expression of the 1298 bp reporter construct was observed in the central cylinder within the root tip, namely, in the initial cells and in the cell files of the pericycle and stellar parenchyma. GUS activity was absent both from the elongation zone and from the incipient lateral root primordia. The 2012 bp reporter construct was expressed throughout the central cylinder including the elongation zone, and in lateral root primordia. Thus, both the 1298 bp and 2012 bp reporter construct show tissue-specific expression in the central cylinder, and the GUS activity in the elongation zone and in lateral root primordia probably connected to a *cis*-acting elements located between 1298 and 2012 bp from the transcriptional start site.

In addition gene expression manipulation experiments were carried out. An RNAi construct (pK7GWIWG2(II)-CpALF4) was prepared to study the function of the *ALF4* in lateral root initiation in cucurbits. Expression levels of *ALF4* were analyzed in individual transformants by quantitative real-time RT-PCR. In general, *ALF4* expression levels were decreased to 20-30 % of wild type activity.

The data obtained from the expression analysis suggest that *ALF4* expression is specific to the central cylinder. Analysis of transgenic hairy roots containing an *ALF4* RNAi construct as well as a *pALF4::Egfp-gusA* construct showed that although GUS activity was observed in the incipient lateral root primordia and the relative expression of *ALF4* was decreased, numbers of lateral roots were not changed in transgenic plants compared to the wild type. This result can be explained by the difference between two main strategies of lateral root initiation. In the case of Arabidopsis, lateral root initiation takes place above the elongation zone. Pericycle cells that are able to form lateral root primordium, re-enter the cell cycle (G1 phase) after a temporary cell cycle arrest. In this system, ALF4 seems to be a key regulator of the transition from cell cycle arrest to the first formative division. On the other hand, in cucurbits the initial steps of lateral root initiation take place within the parental root meristem and surrounded with proliferating cells in pericycle and other tissues. We propose that due to the lack of a temporary cell cycle arrest in pericycle cells, ALF4 does not have a target for interaction.

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Control of PM H^+-ATPase activity and lateral membrane allocation of this enzyme as one of the mechanisms of plant hormones involvement in the regulation of petunia pollen tube growth

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Earlier, the results of the studies directed to elucidating the hormonal status of petunia pollen-pistil system provided evidence for a possible involvement of plant hormones in signal cascade of events of the progamic phase of fertilization. However, the mechanisms of their action as well as a possibility of hormone functioning as signal molecules in the pollen-pistil system remain so far to be unstudied. In the present work, the data indicating auxin (IAA) and abscisic acid (ABA) involvement in the stimulation of petunia pollen tube germination and growth were obtained. It has been established that this effect of the plant hormones is accompanied by certain modulation of both the activity and lateral localization of the H^+-ATPase in the plasma membrane (PM) of male gametophyte. As judged by the increase in its intracellular pH (pH_c) and the PM hyperpolarization, the activity of the enzyme in question was elevated under the action of IAA and ABA. The hormone-induced modulation of lateral allocation of the H^+-ATPase in the PM was expressed in redistribution of this enzyme into subapical region of pollen tube plasmalemma. Since to date it has been established that the PM H^+-ATPase, known energizing the PM creating on it $\Delta\mu_{H^+}$ as a driving force for transport into the plant cell of various ions and metabolites, plays a central, dominant role in the regulation of polar growth of PTs, there are good reasons to expect a potential importance of plant hormones in this regulation, especially if to take into account the above results. Both the IAA- and ABA-induced increase in pH_c and the membrane potential of pollen tubes were shown to be mediated by Ca^{2+} ions influx into them from the external medium and the generation of reactive oxygen species (ROS) most likely triggered by the activity of PM NADPH oxidase. Involvement of Ca^{2+} and ROS in the hormonal signal transduction in petunia male gametophyte germination and growth implies that calcium and redox signaling may underlying the mechanisms used by plant hormones to control the processes of the progamic phase of higher plant reproduction. The hormonal control of petunia pollen tube PM H^+-ATPase revealed here is in accordance with recently offered hypothesis on the important role of this enzyme in integration of external and cytosolic signals from signal transduction and metabolic pathways and their translation to essential nodes in the signaling network of pollen tubes.

The role of secondary active cytokinin transport in the control of their delivery from roots to shoots

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A lot of data shows the presence of cytokinins in xylem sap and changes in their concentration when the root environment alters. The decline in cytokinin supply from stressed roots was accompanied by retardation of leaf growth, and an acceleration of leaf senescence. Since these processes can be influenced by cytokinins, these stress effects were attributed to the reduced flow of these hormones from the roots. This conclusion draws attention to the mechanisms controlling cytokinin delivery from roots to shoots. Most of researchers attribute the changes in cytokinin export to the shoots to the relative levels of synthesis and decay in the roots. However there is at least one more mechanism that may be involved in the control of the process. It is in the regulation of cytokinin transport per se. To address this problem we studied the distribution between shoots and roots of cytokinin zeatin supplied to roots in the nutrient medium of 1-week-old wheat plants. As expected, treatment with zeatin increased cytokinin content in the plant as a whole. However, the extent of cytokinin accumulation was much greater in roots than in shoots. Immunohistochemical localization of cytokinins showed that zeatin treatment intensified

immunostaining of cells in the central cylinder of the roots. Thus there seemed to be a mechanism enabling cytokinins to be retained in root cells and preventing their outflow from roots to xylem sap.
We tested the idea that retention of zeatin in roots may be due to its active uptake by root cells. Transporters of adenine derivatives have been previously identified in root cell membranes (Burkle et al., 2003). They are capable of active zeatin transfer into cells energized by a proton gradient across membrane. Accordingly, the use of protonophores known to destroy proton gradients inhibits active zeatin uptake by root cells. So, we used such a protonophore to test if active zeatin uptake by cells could explain the accumulation of cytokinins in root cells and inhibition of their flow to the shoot via xylem.
Treatment with a protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) decreased the level of cytokinins not only in roots given zeatin but also in those not treated with this hormone. The effect was accompanied by an increase in the concentration of cytokinins in xylem sap and their content in the shoots. Immunohistochemical staining for cytokinins was less intense in root cells of the central cylinder when the protonophore was applied. Thus inhibition of active cytokinin uptake released the block resulting in lower cytokinin accumulation in root cells and an increased discharge flow to the shoots.
We were interested to test if the level of activity of cytokinin uptake by root cells can change under stress conditions. If true, such a mechanism could regulate cytokinin export to the shoot. To test this we compared the effect of protonophore inhibition of active cytokinin uptake on accumulation of exogenous cytokinins in roots of wheat plants exposed and not exposed to salinity. Salinity itself resulted in greater accumulation of exogenous cytokinins in roots as compared to control plants not exposed to NaCl. Inhibition of active cytokinin uptake was found to decrease the level of cytokinin accumulation in roots, the extent of decline being greater in salt-treated plants. As a result, root cytokinin content in salt-treated and control plants was leveled off by protonophore suggesting that greater hormone accumulation under salinity was due to higher activity of inward cytokinin transporters.
Similar results were obtained for tobacco plants exposed to heat shock treatment. High temperature is known to inhibit cytokinin export from the roots to shoots. This may be due to an accumulation of cytokinins in roots of heat-shocked plants. Again treatment with protonophore leveled off cytokinins in roots of heat-shocked and control plants suggesting that the difference between them arose from higher activity of inward cytokinin transport in root cells of the stressed plants.
Thus changes in cytokinin uptake by root cells may provide a mechanism controlling cytokinin distribution between roots and shoots thereby regulating their functions.

Oligosaccharin is an effective messenger of the IAA-induced formation of adventitious roots

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The lateral roots formation is a sequence of well-characterized stages. The main regulator of rooting is auxin, which affects the formation of both the lateral and adventitious roots. But a search for other endogenous regulators involved in this process is still ongoing. We obtained the oligosaccharin from pea seedlings, which increased the IAA-induced number of roots on both the segments of buckwheat's hypocotyl (*Fagopyrum esculentum* Moench) and explants obtained from leaves of transgenic (rolB-GUS) tobacco *Nicotiana tabacum* L. cv Petit Havana when added together with a hormone in culture medium. The greatest effect was reached at short pre-treatment of explants by oligosaccharin (5 μg/ml) before the hormone addition. The optimal time of pre-treatment depends on the model system and ranged from 5-24 hours to 1-2 hours for buckwheat's hypocotyl explants and segments of tobacco leaves, respectively. Adding the oligosaccharin after IAA did not affect the number of roots induced by hormone. Using explants from leaves of transgenic tobacco containing the GUS reporter gene under control of the auxin-inducible promoter of rolB gene allowed to observe the response of explants to hormone at the early stages of root formation. The dynamics of glucuronidase activity (GUS-activity) at a concentration of 3 μM IAA, characterized by the presence of two peaks, which are likely to be related to the key points in the process of the formation of roots, when the tissue explants respond to auxin. Pre-treatment of explants from leaves of tobacco by oligosaccharin caused not only the increase, but also the acceleration of the response of GUS activities, namely shift of the first peak to the left; to the beginning of explants cultivation. The position of the second peak didn't change. The histological analysis of tobacco hypocotyl segments showed that the first peak coincided with the formation of 5-6-layer primordia, which did not always develop later to mature root. The second peak, most likely, coincides with a stage of the almost mature rootswhich itself is an auxin producer. The obtained data specify that oligosaccharin action precedes hormone action at the early stages of root formation.

Further histological and biochemical researches with the use of methods of molecular biology will allow to better understand mechanism of the IAA-induced roots formation and to define the role of endogenous oligosaccharinin in this process.

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The role of glucose in the modulation of the auxin signaling pathway during the formation of Karelian birch figured wood

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Betula pendula Roth is an intriguing study object through which the mechanisms of trunk tissue formation can be investigated, because the species comprises forms differing notably in wood texture: common, straight-grained silver birch (SB, *B. pendula* var. *pendula*) and Karelian birch (KB, *B. pendula* var. *carelica*), which has figured wood. The percentages of structural elements in the wood of these two birch forms are the following (SB / KB): fibrous tracheids - 62.6% / 21.1%, vessels – 23.7% / 1.9%, parenchyma cells – 13.7% / 77%. Thus, parenchyma cells in figured Karelian birch wood are far more numerous. Furthermore, the orientation of vessels and fibers in KB wood is disrupted up to their 90° turning or formation of spiral figures.

The phloem in KB functions in the situation of sucrose excess, which induces high apoplastic invertase activity (2.5 that of SB) (Galibina et al. 2015). In the zone of xylem (wood) growth and differentiation the activity of apoplastic invertase in KB is 4 times higher compared to SB. The reaction products are glucose (Glc) and fructose. Consequently, the level of hexoses in the apoplast of phloem, xylem and, accordingly, cambial zone in KB is much higher than in SB.

Cell elongation and spatial orientation depend on the direction of auxin (Aux) transport. Directional Aux efflux from a cell is facilitated by auxin transport efflux carriers, PIN proteins. It has been demonstrated for the roots of *Arabidopsis thaliana* seedlings that elevated levels of Glc can alter the expression pattern of PIN proteins in terms of their greater accumulation in lateral cell walls (Mishra et al. 2009). As a consequence, the direction of cell growth changes, causing cells (roots) to curve. Increasing Glc concentration also induces root waving and coiling (Singh et al. 2014). The data show that the disruption of spatial orientation of the xylem structural elements in KB can be caused by a change of Aux flow direction due to changes in the spatial expression of PIN-proteins under the influence of elevated levels of Glc in the apoplast.

Rising concentrations of Glc lead to an increase in the expression of Aux biosynthetic YUCCA gene family members (Mishra et al. 2009). This effect of Glc is consistent with a higher (1.8-fold) content of Aux in cortex tissues in KB compared to SB (Schetinkin 1987). However, the Glc-induced increased Aux synthesis has no stimulating effect on Aux-induced gene expression level since Glc at the same time downregulates Aux receptor TIR1. The induction of a number of Aux inducible genes was found to be reduced upon application of exogenous glucose (Mishra et al. 2009).

The formation of tracheids and vessels is induced by Aux (Aloni 2015). There is a mechanism of Aux conversion into inactive (conjugated) state in plants. Conjugate synthesis can occur with a participation of UDP-Glc (Ludwig-Muller 2011). UDP-Glc is a product of enzymatic conversion of sucrose (sucrose + UDP ↔ UDP-Glc + fructose) or Glc-1-phosphate (Glc−1−P + UTP ↔ UDP-Glc + PPi). Glc-1-P can be obtained from hexoses formed by cleavage of sucrose by invertase (Kleczkowski et al. 2010).

The large amount of parenchyma in the wood of KB indicates a change in the hormonal status of xylem differentiation zones. Areas where dense figured pattern is forming in KB wood contain ca. 4 times more bound Aux than trunk areas with poor figure (Schetinkin 1987). 1987). One can surmise that the formation of parenchyma cells in KB wood can take place as a result of Aux conjugation in the presence of sucrose (Glc) excess in the cambial zone.

The pathway of cell death (vessels and fibers) and the preservation of live protoplast (parenchyma cells) correlate with different fatty acid compositions of membrane lipids. In the first case the share of saturated fatty acids in the composition of phospholipids and glycolipids increases indicating a deterioration of the functional characteristics of cell membranes. In the latter case the share of unsaturated, mainly dienoic fatty acids, increases reflecting a rise in the activity of cell membranes. During the periods with the highest rate of metabolism, the content of polyunsaturated, mainly trienoic fatty acids, increases in membranes.

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The lipoxygenase pathway in *Poaceae* plant roots

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Different biotic and abiotic stresses activate lipoxygenase signaling system in the eukaryote cells. As a result a wide spectrum of oxylipins is produced, the products of polyunsaturated fatty acid oxidation. Plant oxylipins constitute a family of bioregulators involved into the cell signaling and defence. These products are biosynthesized through the oxidative metabolism of polyenoic fatty acids controlled by lipoxygenases. There are a number of known routes of the plant lipoxygenase cascade. The primary lipoxygenase products, fatty acid hydroperoxides,are further metabolized by a few major enzymes. These enzymes belong to CYP74 family of 450 macrofamily. There are four main types of CYP74 enzymes: allene oxide synthase (AOS), hydroperoxide lyase (HPL), divinyl ether synthase (DES) and epoxy alcohol synthase (EAS).
The present report is concerned with detection of the profiles of oxylipins in *Poaceae* plants roots. The profiles were examined by gas chromatography-mass spectrometry. Incubations of linoleic or linolenic acids with cell-free preparation from wheat (*Triticum aestivum* L.), sorghum (*S. occidentocuresicum* Jak., *S. sudanense* Jak., *S.chinense* Jak.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), millet (*Panicum miliaceum* L.) roots revealed the presence of 13- and 9-lipoxygenase and HPL, AOS, EAS activities. Divinyl ether synthase (DES) products were not detected in the roots. The HPL cascade produces a diversity of oxylipins, including the compounds: azelaic acid, traumatic acid. The predominant product of AOS was identified as *cis*-12-oxo-10,15-phytodienoic acid, *α*-ketol and 10-oxo-11-phytoenoic acid (minor product). Apparently, the role of oxylipins in developing roots associated with the regulation of ontogenesis and protective functions. For example, azelaic acid is known for its antimicrobial activity. The roots of seedlings selected *Poaceae* plants have complex oxylipin profile, there are also unknown products. Continued research will provide fundamental knowledge about the signaling lipoxygenase pathway economically important crops, mechanisms oxylipins biosynthesis, including - compounds responsible for plant adaptation to unfavorable factors.
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Cytokinin signaling system: new developments

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Cytokinins, classical plant hormones, are *N*6-substituted adenine derivatives. They were discovered yet in the mid-50s of the 20th century, but up to the next century the mechanism of their action remained unknown. It was only in 2001 when cytokinin receptors were ultimately identified. At present it is commonly accepted that cytokinins are recognized by hybrid sensor histidine kinases which are large-sized transmembrane proteins. Generally, the receptor consists of 3 main modules: sensing module at the N-terminus, catalytic module in the middle and receiver module (or domain) at the C-terminus of the protein. The signaling chain from the receptor to primary response genes is based on His-Asp phosphorelay and includes two more proteins, phosphotransmitters and transcription factors, the latter referred to as response regulators of B-type. This report will present data mainly from our laboratory on the structure and properties of proteins involved in cytokinin signal transduction.
In the sensing module of the receptor, new highly conserved structural elements were found, evidently playing an important role in receptor functioning. Also highly conserved consensus motifs have been revealed in the downstream transmembrane helix which transmits the signal from the sensor module to the catalytic one. By means of bioinformatic approach, virtual 3D-structures were built of every module of several cytokinin receptors. Also the receptor-phosphotransmitter complex was modeled allowing to identify amino acid residues critical for the tight interaction of these proteins.
The ligand-binding properties of cytokinin receptors were extensively studied. According to their preference to natural cytokinins, the receptors were classified as iP-type- or tZ-type ones. Receptors of iP-type reside predominantly in the root while receptors of tZ-type mostly in the shoot. Taking into account that root-derived cytokinins transported to the shoot are mainly of tZ-type, and shoot-derived cytokinins are mainly of iP-type, a general model for cytokinin circuit and action in the whole plant was

introduced. This model is useful to explain important features of the cytokinin regulatory system. Recently, we have developed a new plant assay system enabling to characterize receptor properties under the conditions close to natural ones. Using this assay we have demonstrated that only cytokinin bases but not cytokinin ribosides possess genuine hormonal activity. This allowed to specify the notion "cytokinin" and correctly determine natural representatives of this hormone family.

For ligand-receptor binding studies, not only natural cytokinins but numerous artificial cytokinin-like ligands were employed. These studies showed an overall clear positive correlation between the affinity of ligands to receptor and their hormonal activity. However, some unique ligands were also found, ones possessing anticytokinin activity, and others specific for only certain receptors. These unusual ligands may serve as new tools for deciphering the molecular aspects of hormone recognition and receptor activation.

One more direction in our work is a study of the subcellular localization of receptor and phosphotransmitter proteins and their complexes. Several methods were used including BiFC (bimolecular fluorescent complementation), isotop labeling, sucrose gradient fractionation and *in vitro* phosphotransfer. Results of all employed methods indicated that the bulk of cytokinin receptors reside in membranes of endoplasmic reticulum (ER), though the occurrence of a small receptor fraction in plasmalemma is not excluded. By contrast, phosphotransmitters are distributed between cell nucleus and cytoplasm, except a small fraction, bound to receptors at the ER. The data on receptor subcellular localization led to the modification of the general schema of cytokinin action in the cell.

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Crosslink between early auxin transduction pathway involving AXR1 and the regulation of IAA level in *Arabidopsis* plants

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AXR1 encodes an E1-like protein which interacts with E1 C-terminal related 1 (ECR1) protein to activate the RELATED TO UBIQUITIN protein (RUB) for conjugation to cullins. Activity of AXR1 leads to neddylation of SCFTIR1-ligase by RUB, which is required for further poly-ubiquitination of AUX/IAAs for proteasome-dependent degradation and thus it supposed to be a key player in auxin signal perception and transduction. Proteolysis of Aux/IAA transcriptional repressors releases auxin response factors and initiates auxin-inducible gene transcription. Since *axr1-3* mutant with altered auxin sensitivity differ in growth and development from the wild type seedlings, we suppose that some components of phytohormone biosynthesis, conjugation, transport or degradation might be affected. Therefore we performed the complex analysis of growth, development, endogenous free IAA concentration and expression of genes, encoding enzymes of auxin biosynthesis (*TAA1* and *YUC* family), conjugation (*GH3* family) and transporters (*AUX1*, *PIN* and *ABCB* family) in shoots and roots of wild type (wt) and *axr1-3* mutants of *Arabidopsis thaliana* plants, grown on medium without or with addition of exogenous auxins (natural auxin IAA - indole-3-acetic acid or its synthetic analogue 1-NAA -1-naphtalenacetic acid). Advanced high-density and low volume microfluidic quantitative PCR platform Fluidigm and methods of multivariant statistics were implicated for gene expression analysis. Exogenous IAA treatment led to free IAA accumulation only in shoots of wt plants. Revealed alteration in free endogenous IAA concentration in shoots and roots of auxin resistant *axr1-3* mutant correlated with modulation of expression of *TAA1* and number of *YUC*s genes encoding enzymes of auxin biosynthesis. Since auxin degradation pathways are poorly characterized and no genes and/or enzymes responsible for IAA oxidation in plants have been identified yet, the genes expression analysis for this process is missing. Thus, in order to assess the contribution of IAA degradation process in the regulation of intracellular auxin homeostasis, we analyzed the IAA-oxidase activity. Based on our experimental data we suggest the link between gene expression, auxin concentration and plant development in *A.thaliana* wt plants and mutants shoots and roots.

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The role of oxypilins produced in allene oxide synthase and hydroperoxide lyase branches in abiotic stress tolerance

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Oxylipins are signaling molecules formed enzymatically or spontaneously from oxygenated fatty acids in all aerobic organisms. In plants oxylipins regulate many aspects of growth, development, and responses to environmental stimuli_ENREF_24. Allene oxide synthase (AOS) and hydroperoxide lyase (HPL) are two dominant branches of oxylipin biosynthesis pathway in plants, competing for a common substrate - hydroperoxide of linolenic acid. AOS branch metabolites, 12-oxophytodienoic acid, jasmonic acid and derivatives, perform a broad range of regulatory functions, including regulation of growth, development, fertility, and stress responses. HPL metabolites, aldehydes, aldoacids and their derivatives, possess antifungal and antibacterial properties, are involved in plant defense against insect and pathogens. The role of metabolites produced in AOS and HPL branches in biotic stress responses has been intensively studied, whereas their role in plant adaptation to abiotic stress conditions is underestimated. Our current research is focused on elucidation of functions of AOS and HPL metabolites in plant tolerance to abiotic stress conditions, such as wounding, drought, waterlogging, suboptimal light and temperature. To uncover functions of studied metabolites in abiotic stress responses we use genetically modified Arabidopsis plants with altered oxylipin profile. Lines are generated in the wild type Colombia-0 background, depleted of functional *HPL* gene due to the deletion in the first exon of the gene. T-DNA insertion mutant in *AOS* gene in Columbia-0 (*aos* plants) is depleted of metabolites of both braches, AOS and HPL. Finally, we have plants expressing rice HPLs in Colombia-0 wild type and *aos* background. These lines were tested for the tolerance to different abiotic stress conditions. Using this approach we could identify 12-oxophytodienoic acid as a drought-responsive regulator of stomata closure functioning most effectively together with ABA. We also found that plants overexpressing HPL better tolerate waterlogging conditions, when roots experience severe hypoxia, and recover faster after removal of excessive soil water, when re-oxygenation occure. Analysis of physiological parameters, gene expression and primary metabolites of plants with altered oxylipin profile under normal and stress conditions revealed specific molecular and physiological properties of HPL-overexpressing plants underlying waterlogging stress tolerance.
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An ARF7/ARF19-regulated kinase controls calcium-dependent tip growth in *Arabidopsis*

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In higher plants, tip growth lies at the basis of root functioning (root hairs) and successful fertilization (pollen tubes). Multiple key players involved in root hair and/or pollen tube development have been identified. However, an integrated view of the tip growth process is often still lacking, and the interconnectivity of known pathways is heavily underestimated. For instance, evidence points towards a central role of auxin signalling in root hair and pollen tube morphogenesis. However, not a single tip growth specific gene has been reported to be a direct target of Auxin Response Factor (ARF)-regulated signal perception. More so, whereas an interdepended functioning has been attributed to the maintained

presence of cytosolic tip-focused calcium oscillations and cell wall composition, actual scientific data to support this has been lacking.

Our findings report on the characterization of ERULUS, a tip growth specific ARF7/ARF19-regulated receptor like kinase with a distinct role in calcium and cell wall signalling in both root hair and pollen tube growth. Our data shows that the *eru* null mutation results in extracellular calcium-dependent perturbation of pollen tube and root hair growth dynamics. ERULUS regulates fertilization efficiency and is involved in the fine-tuning of calcium oscillations in the growing pollen tube tip. The latter was shown by high resolution temporal analysis of Yellow Cameleon 3.6 (YC3.6) dynamics. MicroFT-IR followed by Immunolabelling illustrated that the cell wall of *eru* root hairs exhibit aberrant homogalacturonan methyl-esterification degrees, whereas cellulose deposition was unaffected. Moreover, *ERULUS* transcription is specifically affected in several pectin perturbed mutants. Next, we found that ERULUS is at least involved in the auxin regulation of root hair development, since its transcription is temporally regulated in an ARF7/ARF19-dependent manner. We also showed that ERULUS functions downstream of the key tip-growth transcription factor RHD6, and the small GTPase ROP2 which regulates microtubule organization. As such, ERULUS was identified as a key auxin-regulated component of the tip growth machinery, and an integral part of several interconnected tip growth pathways.

Analysis of expression of marker "senescence genes" and localization of gibberellins in pea (*Pisum sativum* L.) symbiotic nodules

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The studies of the aging of nitrogen-fixing nodules are of the great interest due to possible important practical application. The delay of nodule senescence could improve the crop yield by limiting the use of chemical fertilizers.

In this study the wild-type SGE and a series of symbiotic mutants SGEFix$^-$-1 (*sym40*), SGEFix$^-$-3 (*sym26*) and SGEFix$^-$-7 (*sym27*) characterized by the premature degradation of symbiotic structures and early senescence of nodules were used (1, 2).

The genes encoding cysteine (*PsCyp1*, *PsCyp15a*) (3, 4) and thiol (*PsTPP*) (5) proteases, transcription factor bZIP (*PsATB2*) (6), gibberellin 2-β-hydroxylase (*PsGAOx2*) (4, 7), ACC synthase and oxidase (*PsACS2*, *PsACO1*) (8, 9) and aldehyde oxidase (*PsAO3*) (10) were selected as the marker "senescence genes" of pea nodules.

An evaluation of transcript abundance of all selected genes was shown via real-time PCR during the aging of nodules of wild-type and mutant lines. In 4 week after inoculation mRNA levels of all analyzed genes were significantly higher in early senescence mutant nodules than in the active nitrogen-fixing wild-type nodules.

To identify expression pattern of tested "senescence genes" in infected cells from nitrogen-fixation and senescence zones of wild-type and SGEFix$^-$-7 (*sym27*) nodules laser capture micro-dissection was carried out. The enhancement of *PsCyp15a, PsTPP, PsATB2, PsGAOx2, PsAO3* and *PsACO1* expression levels were observed with an increase in degradation degree of nodule cells.

Immunolocalization of gibberellic acid using laser confocal microscopy was performed for all analyzed genotypes. The intensity of GA labeling was decreased in the senescence zone of wild-type and mutant nodules.

Thus, the positive regulation of pea nodule senescence with ethylene and abscisic acid, the active effect of cysteine and thiol proteases, and transcription factor bZIP in nodule aging were demonstrated. The possible negative regulation of pea nodule senescence with gibberellic acid was also shown.

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Water relations in ABA deficient barley mutant and its parental cultivar under increased temperature

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Plant ability of maintaining transpiration is important for their cooling at increased temperature. This effect is impossible without adequate water uptake dependent on tissue hydraulic conductivity. An increase in the hydraulic conductivity permitted wheat plants to maintain high level of transpiration without reducing water content in leaf tissues at air warming (Kudoyarova et al., 2011). Distribution of abscisic acid (ABA) between wheat shoots and roots seemed to be involved in this, the assumption being based on the literature about the ability of this hormone to increase the hydraulic conductivity through changes in the activity of aquaporins. To test the hypothesis about a dependence of hydraulic conductivity on ABA level, we compared responses to the increase in air temperature in original barley plants (Steptoe) and mutant AZ34 with a reduced ability to accumulate this hormone.

Barley plants were grown at 24 °C for 7 days and then air temperature was increased by 4 °C. Osmotic hydraulic conductivity of plant roots was calculated according to the equation: $Lpr = Jv/\Delta\Psi\pi$, (2) where Lpr—root hydraulic conductivity, Jv—volume of xylem exudate transported through the excised roots, $\Delta\Psi\pi$—difference between osmotic pressure in the xylem sap and nutrient solution. ABA content was determined by the modified immunoenzyme analysis (Veselov et al., 1992). Immunohistochemical localization of PIP2 aquaporins was carried out using polyclonal antibodies for HvPIP2s raised in rabbits against synthetic oligopeptides corresponding to the amino acid sequences in the N- region of HvPIP2 aquaporins.

The rate of transpiration before temperature increase in AZ34 plants was 1.5-fold higher than in Steptoe plants. Air warming was accompanied by activation of transpiration by 30 and 20% in Steptoe and AZ34 plants, respectively. The heating did not reduce leaf water potential of original wild-type plants, whereas in AZ34 leaves it decreased by 0.3 MPa. Hydraulic conductivity was higher in cv. Steptoe and increased after air warming, whereas in AZ34 roots such dependence on temperature was absent. Quantitative estimation of immunostaining for PIP2;2 auqaporins with the help of ImageJ program showed a significant increase in the root cells of Steptoe after air warming paralleled by an increase in hydraulic conductance, while no increase was detected in the case of Az34. The content of ABA decreased in the leaves and increased in the roots of cv. Steptoe with the air warming. In the leaves and roots of AZ34 plants, ABA content was lower than in cv. Steptoe plants and was unchanged after temperature increase. The elevated level of transpiration in mutant plants was evidently related to a decrease in ABA content in them, whereas activation of transpiration in Steptoe plants could be facilitated by a decrease in the ABA content at increased air temperature. Steptoe plants were capable of maintaining leaf water potential at air warming, while it remained low in AZ34. Maintenance of leaf water potential by Steptoe at increased temperature was due to elevated hydraulic conductivity. This pattern is characteristic of the plants with a normal ability to synthesize ABA (cv. Steptoe). These results correspond to literature data showing an increase in hydraulic conductivity brought about by the increase in the activity of water channels with the elevation of transpiration (Clarkson et al., 2000). ABA is capable of increasing root hydraulic conductivity due to its action on aquaporin activity. ABA-deficient AZ34 mutant was used to allow testing the hypothesis about the role of this hormone in the maintenance of leaf water potential at increased air temperature. As distinct from cv. Steptoe plants accumulating ABA in the roots at temperature increase, mutant plants with the low ABA content could not maintain water potential at air warming. Obviously, this occurred because the abundance of PIP2;2 aquaporin did not change and hydraulic conductivity in mutant plants did not increase. Thus, our results indicate that the regulation of hydraulic conductivity and abundance of aquaporins in the root cell membranes at temperature increase is related to ABA accumulation in the roots. Our results indicate that plant ability of increasing hydraulic conductivity is important for the functioning of involved mechanisms. This process is possible when plants can accumulate ABA.

Crosstalk of Jasmonates and copper in growth and photosynthetic efficiency regulation of *Cajanus cajan* seedlings

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Plant hormones methyl jasmonates (Me-JA) and Jasmonic Acid (JA) jointly called jasmonates (JAs) constitute a small group of related molecules originated from Linolenic Acid via LOX and AOS pathway. JAs are signaling molecules which control a number of physiological activities such as seed germination, growth, trichome formation, tuber formation, senescence and stress responses through signal transduction cascade. Within plant cell signal transduction pathway operates in complex web interactions to regulate various responses. These interactions are termed as cross talk regulations of different signaling pathways. These signal transduction pathways may also interact with different metal ions which are integral part of metabolic machinery. Copper (Cu) is one such metal ion which is responsible for integrity of proteins involved in photosynthesis. In the present study the effect of Me-JA (1µM) and JA (1nM) has been studied on the growth, photosynthetic efficiency and Saccharides of 15 days old seedlings of *Cajanus cajan* under copper (Cu) stress. *C. cajan* seeds were treated with Me-JA (1µM), JA (1nM) and grown in 5mM copper sulphate solution in petriplates for 15 days. Cu deteriorated growth in terms of root length and shoot length but showed amelioration when JA or Me-JA was signalized in the system prior to Cu exposure. Different components responsible for photosynthesis such as pigments of antenna complexes viz chlorophyll a, b, total chlorophyll and carotenoids behaved synergistically in Cu and JAs alone or in combination. JAs also showed harmonious effect which was although partial on photosynthetic efficiency amelioration of *C. cajan* seedlings with or without Cu. On one hand qP was symbiotically enhanced in JAs and Cu treatments but Fv/Fm showed antagonistic behaviour in these treatments. Saccharides are the product of actual photochemistry occurred in the plant cell which showed synergism in JAs and Cu treatments alone and in combination. Overall we can conclude that JAs have some crosstalk at any of these levels of interaction responsible for cross regulation of plant growth and development in *C. cajan* seedlings under JAs and Cu treatments.

The endorhyzobacteria *Bacillus licheniformis* isolated from *Dactylorhiza maculata* (L.) Soó (Orchidaceae Juss.) as the phytohormone producers

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The aim of this research was to estimate the plant growth promoting (PGP) abilities of two strains isolated from *Dactylorhiza maculata* tubers and belonging to the same species: *Bacillus licheniformis* K and *B. licheniformis* E5.

The capability to produce indoleacetic acid was studied in periodic growth dynamics of the isolated cultures, using Czapek medium with tryptophan as a precursor of phytohormone that is indole-3-acetic acid as was described earlier (Shekhovtsova et al., 2012).

The curve of *Bacillus licheniformis* K growth based on the changes in optical density of bacterial suspension at 590 nm (D_{590}) proves to be closest to the classical one. On it there could be identified two phases: the phase of growth and the phase of culture die-off. We've observed active growth of bacteria for three days. During this time value of D_{590} increased by 15.2 times (from 0.44 to 6.68). Culture multiplied at maximum specific rate (µ) 1.45 day^{-1} during the first 24 hours, then µ gradually decreased. Mean specific growth rate equaled 0.91 day^{-1}. Later we've observed that D_{590} was decreasing irregularly – the fact reflecting the ability of bacteria from genus *Bacillus* to switch from oxidative metabolism to fermentative metabolism, as well as their capacity to form spores. Generally die-off of the culture occurred at mean specific rate $k = 0.12\ day^{-1}$.

Production of indoleacetic acid (10.5 mcg/ml) was recorded during the second day of the culture *B. licheniformis* K growth. Maximum concentration of the phytohormone in cultural medium (15.0 mcg/ml) coincided with the biomass maximum. Later decrease in D_{590} by 37% was accompanied by gradual decrease of the concentration of IAA to the 26% by the end of the observation period.

The second strain of *B. licheniformis* E5 proved to be less adapted to growth on Czapek medium. Increase in biomass was less significant: D_{590} increased by 10.6 times. Also in this case the culture was observed

growing for 3 days, but this process was divided into two stages. During first 24 hours growth occurred with maximal specific rate 1.77 day^{-1} and on the third day at the rate $\mu = 0{,}55$ day^{-1}. During the second day the growth rate was very slow (0.04 day^{-1}), which was apparently related to the adaptation of the culture to tryptophan utilization and to indoleacetic acid synthesis, because the phytohormone appears in the cultural medium only on the third day. Therefore bacteria *B. licheniformis* E5 should be rated as moderate producers since the maximum indoleacetic acid output reached 4.2 mcg/ml only. Maximum concentration of exometabolite coincided with biomass maximum. Side by side with D_{590} decrease there occurred similar decrease of indoleacetic acid in the medium. By the end of the observation period for both parameters it reached 1.7 times.

Differences in periodic growth dynamics of the two strains *Bacillus licheniformis* E5 and K discovered here are consistent with views expressed by different authors [Etesami et al., 2009; Patten et al., 1996] concerning complex nature of IAA synthesis regulation for different bacteria. Apparently, strain E5 is capable of producing tryptophan only by induction while culture K uses several ways to synthesize the exometabolite.

It is known that the production of IAA by growth stimulant bacteria when adding tryptophan to the cultural medium varies from 5 – 10 up to 100 – 200 mcg/ml [Halda-Alija, 2003]. Therefore, the g. *Bacillus* cultures we've isolated from *D. maculata* tubers can be rated as moderate IAA producers only. However, the results we've obtained are consistent with the research of Tsavkelova et al. (2005; 2007) who has found that the IAA level produced by g. *Bacillus* bacteria isolated from roots of terrestrial orchids and substrate roots of epiphytes varied depending on the strain and was within the range of 4 to 55 mcg/ml. Our data also corroborate the results obtained by Australian researchers [Strzelczyk, 1984], who've discovered the ability of bacterial strains *B. shaericus* and *B. cereus* associated with roots of Australian orchids to produce IAA.

It is known that numerous microorganism strains of g. *Bacillus*, isolated from rhizospheres of plants of different species are used in agriculture as most active auxin, and specifically IAA producers [Darbyshire, 1993; Chen et al., 2009]. The strains isolated are supposed to be used when growing *in vitro* a rare species of moderate climate orchid – *D. maculata.*

Regulation of jasmonate's signaling components expression in *Cajanus cajan* (L.) Millsp. by exogenous JA, Me-JA and copper

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Jasmonates (JA's) are lipid based signaling molecules involved in modulating number of metabolic processes responsible for plant growth, morphogenesis and stress responses. In present study the effect of methyl jasmonate and Jasmonic acid treatment have been analyzed in 15 days old seedlings of *Cajanus cajan* (L.) Millsp. Seeds primed with Me-JA (1μM) and JA (1nM) were grown in petriplates having 5mM Copper Sulphate for 15 days under controlled conditions of seed germinator and used for gene expression analysis. JA's signaling is mediated by various transcription factors such as *COI1*, *JAZ*, *MYC2*. COI1 is the receptor of JA signaling whose expression was downregulated in Me-JA and JA treated seedlings grown in $CuSO_4$. *JAZ* is the negative regulator of JA signaling which belongs to *TIFY* family of transcription factors and is involved in plant growth and development, phytohormones signaling and various abiotic and biotic stresses. *C. cajan* contains 18 genes which belongs to *TIFY*, *JAZ* and *ZML* subclasses. Relative expression studies of these *CcTIFY* genes showed that *CcTIFY3*, *CcTIFY4*, *CcTIFY5*, *CcTIFY8*, *CcTIFY9* and *CcTIFY16* were upregulated in presence of Cu without JA's. However, in 1μM Me-JA treated seedlings 4 genes viz. *CcTIFY2*, *CcTIFY4*, *CcTIFY5*, *CcTIFY9*, *CcTIFY12* and *CcTIFY13* were upregulated. While in 1nM JA treated seedlings only two genes *CcTIFY9* and *CcTIFY13* were upregulated. *MYC2* which is the master regulator of JA mediated signaling was down regulated in all the treatments. Expression of Calmodulin gene (*CAM*) and *MAPK Kinases* was also analyzed and it was found that these genes are involved in JA's signaling in *C. cajan* when exogenous JA, Me-JA of Cu was given to the seeds or seedlings.

Features of lipoxygenase cascade of different plant species

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Lipoxygenase pathway, a source of biologically active oxylipins, is widespread in plants. The pathway beside lipoxygenases is controlled by the enzymes of CYP74 family, nonclassical cytochromes P450: allene oxide synthases (AOSs), hydroperoxide lyases (HPLs), divinyl ether synthases (DESs) and epoxyalcohol synthases (EASs). Oxylipins include a large number of structurally different compounds: jasmonates, cyclopentenones, aldehydes, aldoacids, alcohols, divinyl ethers, epoxyalcohols, trihydroxyacids etc. Physiological functions of oxylipins are diverse: they participate in regulation of different processes – bloom, aging, response on stress factors, including pathogen attack, wound healing and others. Schemes of plant defense againt pathogens are often complicated and specific. However, functions of many oxylipins remain hypothetical. Thus, suggestion of antibacterial and fungicide properties of divinyl ethers are based on results of experiments on study of gene expression dynamics and oxylipins patterns changes. In our laboratory study of biological action of different oxylipins were performed. Besides, in our researches a number of nonclassical oxylipins with unknown functions and biosynthetic mechanisms was revealed.

At present the CYP74 enzymes are the most widespread in flowering plants. Therefore, for a long time it was believed that the CYP74 enzymes exist solely in flowering plants. This fact was the basis of the concept of recent origin of the family. New data on the presence of CYP74 enzymes in bacteria, fungi, algae and animals provide a basis for the revision of these views. Modern classification is based on the data of CYP74 family and P450 superfamily as a whole is much more complex than originally proposed. Due to appearance at ancient times and changing during long time now lipoxygenase cascade of evolutionally distant organisms varies. The most studied branches of lipoxygenase cascade are AOS and HPL. Jasmonates, aldehydes and aldoacids – products of these pathways – are common for all land plants. Unlike AOSs and HPLs, DESs and EASs are less studied enzymes. For instance, divinyl ethers and corresponding enzymes have been detected in few plant species taxonomically distant from each other. This suggests that this branch is the youngest. However, we studied oxylipins pattern of *Selaginella moellendorffii* which contains the most diversity of divinyl ethers. We cloned and characterized two DESs of *S. moellendorffii* producing different isomers of etherolenic acid. Thus, *S. moellendorffii* has DESs, and at the same time lack jasmonic acid. It means later becoming of AOS branch in the form in which it exists in flowering plants. Before flowering plants there is no reductase in AOS pathway, and this branch ends with reaction of synthesis of cyclopentenone possibly having functions as jasmonate in flowering plants.

We cloned and characterized lipoxygenase cascade enzymes of several high plant species, such as maize, flax, soybean, cucumber, potato, moss *Physcomitrella patens*, *S. moellendorffii* and some others. All these species have different number and set of the CYP74s. AOSs and HPLs exist in all these species, DESs and EASs – only in some of them. Some enzymes have several isoforms, another exist in a single copy. We analyzed dynamics of lipoxygenase cascade gene expression in different conditions. Besides, we studied structural-functional interrelations of the CYP74s by site-directed mutagenesis. And finally, we hypothesized evolutional history of the CYP74 family by itself and within the whole P450 superfamily.

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The hormonal regulation of larix sibirica somatic embryogenesis in culture *in vitro*

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Immature isolated zygotic embryos and megafgamethophytes of *Larix sibirica and L. sukaczewii* were experimentally cultured on AI medium (http://www.freepatent.ru/images/patents/5/2456344/patent-2456344.pdf) added by 2.4-Dichlorophenoxyacetic acid and 6-benzyladenine. The active proliferation of embryonal suspensornal masses (EMS) is observed on the same medium with reduced concentration

of cytokinins. The embryological processes in vitro followed one and the same scheme: elongation of somatic cells and their asymmetric division with formation of initial and tube cells. Immunohistochemical study of hormone localization showed that IAA localized at one of poles of elongated cells. Kinetin was observed in 36% of cells. ABA localized at the periphery of cells.The cells of embryo initial underwent sequential divisions and formed masses of small embryonal cells (embryonic globules). Suspensor-like structures are developed from the edges of embryonic globules. Somatic embryos became mature and germinated by addition of abscisic acid and polyethylene glycol into the medium. 16 long-term proliferating cell lines (3-7) years) and plantlets were obtained in *Larix sibirica.* These lines can produce the number of somatic embryos and plantlets over three years (from 400 to 11000 per 1g of callus fresh weight). ABA accumulation was observed in cells of globular somatic embryos incapable to maturation.
Somatic embryogenesis pass over the strong genetic control. Only single donor trees form embryogenic cell lines and somatic embryos.
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Correlation barley stem growth in plants

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Auxin under environmental conditions conducive to its domination makes sowing spring barley has different stams, productivity decreases. Hormone ethylene carries apical dominance in the prioritization of productive growth of the stem, the tendency to cancel barley lodging of a possible manifestation with the opening of the additional potential of stems in the early growth of the latter internodes. Inhibition of the simultaneous growth of the rape brushes with not generate additional elements of productivity due to spending on growth assimilates unfertilized flowers. There is an adaptive level of productivity, which coordinates balance hormone stimulators and inhibitors in the apex of a real possibility, the crop. When stressful weather conditions it is possible to combine the vertical resistance to barley before the lodging of its possible manifestations and further growth of the gross yield. Adaptive stalks restricts competition in the plant under favorable weather conditions after flowering. The height of the main stem is limited. Ethylene is in balance with the auxin communicates with the weather conditions and corresponds to the actual yield correlation stems of the plant and crop. Effect of inhibitor Initial cells secondary intercalary tissue in the stem height is far less important. Ethylene in the last period of growth internodes tall varieties of steppe type in the period of growth of the upper internodes not only largely reduces the height of the stem, but conveys the formation of the productive elements of the next stalk. Excessively high average temperature and rainfall sufficiency in the steppe zone in the period of intensive growth stems excess auxin in the stem apex of the dominant causes and stretching and twisting the main stalk of barley. A surplus of ethylene in the cold spring to tillering. At a low level of productivity of barley inhibitor is effective in conjunction with the special liquid complex fertilizers, as formation of elements in the plant continues. With an average level of productivity of barley does not require exogenous hormone inhibitor, and an adaptive climate determines the production area of cultivation culture. Inhibition of rape brush does not increase yields and affects only the weight of oilseeds because of the limitation of flowering and abortions apical unfertilized flowers. For culture indeterminate apical dominance Brush spring rape inhibition of irrelevant control the formation of elements of efficiency and effective processing of liquid fertilizer during the action of endogenous growth factors.

Interaction of ethylene and ABA in the control of transpiration in wheat plants under conditions of water deficit

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Information about the effect of ethylene on plants, although extensive, is often contradictory. It is assumed that the ambiguity of ethylene action on plants is related to its interaction with other hormones. By itself, ethylene induces stomata closure, but in combination with its antagonist ABA, ethylene may, in contrast, facilitate stomata maintenance in the open state. The objective of this work was to elucidate the role of ethylene and its interaction with ABA in the regulation of plant water relations at water deficit.

To do this, before watering cessation, wheat plants were treated with the inhibitor of ethylene perception, 1-methylcyclopropene (1-MCP).
In two days after cessation of watering, the rate of transpiration and stomatal conductance decreased only in plants treated with 1-MCP. On the third day, the rate of transpiration declined in untreated plants as well but to a lesser degree than in treated plants. In plants untreated with 1-MCP RWC was reduced as compared with well watered plants, whereas in treated plants it retained on the level of well watered plants. Untreated stressed plants lost their turgor, whereas plants treated with 1-MCP maintained it. Two and three days after watering cessation, plants treated with 1-MCP contained 3.0 - 2.5 times more ABA, respectively, in the leaves as compared with well watered plants. In plants untreated with 1-MCP, the content of ABA in the leaves increased significantly (approximately twice as compared with control) only on the third day after the stop of watering.
During the first two days after the start of experiment, leaf water content in stressed plants was maintained on the level of well watered plants. And at the same time in plants treated with 1-MCP, stomata were closed indicating the role of chemical but not hydraulic signals in the control of stomatal movements. It should be noted that the wheat cultivar used in our experiments (Bezenchukskaya 139) is characterized by a slow stomatal response to water deficit in soil. When sand moisture reached 25%, stomata of this cultivar remained open and a decrease in the rate of transpiration was observed only on the following day. The capability of maintaining stomata in the open state under water deficit conditions is a specific characteristic of some cultivars. In experiments, when ABA content in the plant leaves was reduced artificially, it was found that stomatal opening was related to the low ABA content. At the same time, the mechanism controlling the ABA level in leaves under water deficit remained unclear. Our results indicate a possible role of ethylene in this process. More rapid and significant stomata closure in plants treated with 1-MCP shows that, at water deficit, ethylene favors stomata maintenance in the open state. Earlier, it has been shown that enhanced ethylene production under the influence of ozone counteracts ABA-induced stomata closure (Wilkinson, Davies, 2009). On the basis of experiments with ozone plant treatment, it was suggested that antagonism between ethylene and ABA action on stomata is realized at the level of signaling systems. Our experiments do not exclude such a possibility. However, the explanation may be different, because ethylene effect on the content of ABA was observed under water deficit. We found a more rapid and significant ABA accumulation in plants treated with 1-MCP, and this indicates that ethylene can influence ABA accumulation. It was shown that ethylene can decrease the ABA content in plants (Jackson, 2008), that is in accordance with our results. In our experiments plants untreated with 1-MCP were characterized by slower and smaller accumulation of ABA under water deficit than plants treated with 1-MCP, and this may explain specific responses of their stomata. Collins and co-authors (2008) discussed the fact that, at short-term and moderate drought, plants capable of keeping their stomata open and thereby supporting normal gas exchange and photosynthesis, are characterized by the higher growth rate and productivity. Some fine regulatory mechanism should switch plant strategy from keeping stomata open to their closure dependent on duration and severity of water deficit. Literature data and our results indicate a possible role of interaction between ethylene and ABA in the functioning of this mechanism.

Phenolic acids content in Lupinus angustifolius L. leaf extracts in the presence and absence of bean yellow mosaic virus (BYMV) symptoms

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The pathogenesis of the viral infection in plants is characterized by multidirectional processes.
On the one hand, viruses break normal course of biochemical reactions in plant cells and depress functions of different organs. Furthermore, the active viruses multiplication occur at the expense of depletion of the host plant resources. On the other hand, plants modify their metabolic processes due to activation of the defense mechanisms, and generation of the phytoalexins. The phenolic compounds play an important role in this process.
Bean yellow mosaic virus (BYMV) belongs to the group Potyvirus family Poxviridae. The wide spread of the virus on blue lupine is observed in 10-15 days after aphid flight. Infection is observed from the beginning of plant budding and up to the pod formation.
Changes of the phenolic acids content in the Lupinus angustifolius L. (cv. Rubine, Germany) leaves extracts under the influence of the viral infection are evaluated.
For this purpose the leaves samples were taken from the healthy plants and plants with the typical initially BYMV symptoms under the native infection. The leaf samples from the main stem and lateral branches were taken separately.

At that moment on the healthy plants there were the large dark green leaves and the raceme with the flower buds on the main stem and the small young leaves on the lateral branches.
The plants with BYMV symptoms looked like as follows: the main stem with raceme began to curl into the form of a “Shepherd's crook”, but the leaves on the main stem externally remained unchanged; the young leaves on the lateral branches displayed narrower and deformed leaflets.
The infected plants was detected by external symptoms and by electron microscopy. The analysis of the chlorogenic, ferulic and gallic acids content in methanol extracts of leaves were determined by HPLC
Changes of the phenolic acids content in leaves extracts of plants with BYMV symptoms as oppose of healthy plants were established.
In the plants with BYMV symptoms was found elevated levels of the mean content (from the main stem and lateral branches) of ferulic acid by 3 times (from 3.2 to 9.5 mg / 100g) and gallic acid by 1.8 times (from 6,0 to 10.7 mg / 100g) as oppose of healthy plants. While no significant differences were found in the chlorogenic acid content (0.17-0.20 mg / 100g).
In the healthy plants the ratio of phenolic acids content in the extracts of leaves from the main stem to the leaves from the lateral branches was 1:2,2 for ferulic acid, 1:1,8 for gallic acid, and 7:1 for chlorogenic acid. In the pants with BYMV symptoms the indicators changed exactly the opposite: 6:1 for ferulic acid, 3,4:1 for gallic acid and 1:1,8 for chlorogenic acid.

Redistribution of hormones and growth response of plants to the presence of competitors

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Despite an interest in the problem of the control of growth response to the increase in planting density, nowadays there is only fragmentary information about the role of plant hormones in the perception of the presence of plant neighbors (competitors).
To model competition (planting density) in laboratory experiments we planted one or three lettuce plants in the pots. Observations showed that a decline in transpiration and an increase in shoot/root mass ratio, as well as an increase in specific leaf area (SLA), characteristic for plant response to the light signal, were the fastest lettuce responses to the presence of competitors. Treatment of single and competing plants with inhibitor of ethylene reception (1-methylcyclopropene), inhibitor of auxin transport (naphtylphtalamic acid) and auxin producing bacteria *Paenibacillus illinoisensis* (natural source of rhizosphere auxins) modified the growth response to the presence of competitors in the pots preceded by the changes in concentration and content of hormones.
As a result, it was detected that the decline in the root mass of competing plants as compared to the single plants is associated with the decrease in the concentration and content of auxins, while the increase in SLA and in the proportion of shoot in the plant mass is linked to allocation of auxins to the shoots. This conclusion was supported by experiments with the inhibitor of auxin transport and introduction of auxin producing bacteria. Naphtylphtalamic acid disturbed polar auxin transport thereby decreasing root/shoot mass ratio. Introduction of bacteria into substrate of single plants led to a response similar to the reaction of competing plants to shading, i.e. to an increase in shoot/root mass ratio and in SLA. Accordingly, auxins delivered to the roots are likely to be involved in the regulation of the growth of shoots and leaves under competition for light. No increase in SLA was detected in plants treated with the inhibitor of ethylene perception suggesting necessity of sensitivity to ethylene for manifestation of this growth response. Allocation of auxins to the shoots of competing plants as well as to the shoots of plants with inhibited perception of ethylene preceded inhibition of root growth as compared to single ethylene sensitive lettuce plants. Combination of disturbance of auxin flow to the roots with accumulation of cytokinins in the roots led to even greater decline in the root mass of competing plants that lost sensitivity to ethylene in comparison with ethylene sensitive plants. These results serve as evidence in favor of involvement of ethylene in bringing about the plant growth response to competition due to its effect on distribution of auxins and cytokinins. Effect of ethylene on concentration and distribution of ABA in the plants under competition was less pronounced than its effect on IAA and cytokinins. Inhibition of growth and transpiration in competing plants correlated with accumulation of ABA in the shoots. Consequently, inhibition of accumulation of biomass of competing lettuce plants may result from indirect effect on stomatal closure and ABA-induced inhibition of photosynthesis.
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Prey induced phytohormones and gene expression in carnivorous *Nepenthes*

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Carnivorous *Nepenthes* plants use modified leaves forming pitfall traps to capture and digest prey, mainly insects, for additional nutrient supply. These traps, so called pitchers, contain a plant-derived fluid composed of many hydrolytic enzymes and defense-related proteins. The prey-induced induction of corresponding genes of those proteins and a role for phytohormones in this process was analyzed. Insect prey in the pitchers induced the accumulation of phytohormones such as jasmonates as well as the transcription of studied genes encoding a chitinase 3 and a protease (nepenthesin I), whereas a defense-related protein (PR-1) gene was not induced. Treatment with chitin as component of the insects' exoskeleton triggered the accumulation of jasmonates, the expression of nepenthesin I and chitinase 3 genes similar to jasmonic acid treatment, and induced protease activity in the fluid. The results suggest that upon insect prey catch a sequence of signals is initiated: (i) insect-derived chitin, (ii) jasmonate as endogenous phytohormone signal, (iii) the induction of digestive genes expression, (iv) protein expression. This resembles a similar hierarchy of events as known from plant pathogen/herbivore interactions supporting the idea that carnivory evolved from plant defenses.

SESSION 8

Interactions with Bacteria and Fungi

A potential involvement in cell signaling of symbiosome membrane Ca^{2+}-ATPase from bean root nodule cells based on the mechanism of its functioning

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In eukaryotes, including higher plant cells, key regulators of cytosolic Ca^{2+} level are Ca^{2+}-ATP-ases extruding Ca^{2+} from the cytosol using the energy of ATP hydrolysis. At present, emerging evidence leads to the conclusion that Ca^{2+} ejection activity of these pumps is less important to the total regulation of cytosolic calcium level and their main role is instead the regulation of Ca^{2+} in restricted cytosolic domains in which they interact with numerous important enzymes. On the other hand, in the light of this new concept it is reasonably to expect that Ca^{2+}-ATPase-driven Ca^{2+} accumulation in restricted luminal domains within some intracellular compartments, such as vacuoles and symbiosomes, is of great importance as well. To date, it is known that symbioso-mes as plant-derived membrane compartments in infected root nodule cells responsible for nitro-gen fixation in plants occurring in the course of mutualistic legume root cell-rhizobium symbio-sis are equipped by Ca^{2+}-ATPase functioning in the symbiosome membrane (SM). This enzyme pumps Ca^{2+} ions through the SM from the cytosol of infected cells into the symbiosome space (SS) surrounding nitrogenase-containing bacteroids. According to our recent findings ATP-dri-ven transmembrane translocation of Ca^{2+} appeared to be accompanied by transport of H^+ ions in opposite direction, i.e. efflux of them from symbiosomes. Such a mechanism of SM Ca^{2+}-ATP-ase operation suggests a certain signaling role of this enzyme in symbiosome functioning if to take into account the following evidence. First, both plant vacuolar compartment and the SS contain calmodulin-like Ca^{2+}-binding proteins (Yamaguchi et al. 2005; Lui et al. 2006), with at least one vacuolar such type protein is capable of involving in a Ca^{2+}- and pH-dependent manner in the regulation of tonoplast ionic transporters activity, in particular the activity of Na^+/H^+ antiporter (Yamaguchi et al. 2005). Second, it is evident that thanks to the revealed mechanism of SM Ca^{2+}-ATPase action this enzyme is capable of modulating both pCa and pH within the SS. In this case, the above sensor proteins might be sensitive to this modulation and respond by their binding with any transporters in the symbiosome or bacteroid membranes leading to certain changes in their functioning. As a working hypothesis, we assume that the identified mechanism underlying SM Ca^{2+}-ATPase operation is the important factor determining a signaling role of this enzyme in symbiosomes functioning.

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The role of polyamines in fungal development during *Sporisorium scitamineum*-sugar cane interaction

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Sporisorium scitamineum is a filamentous fungus that causes smut disease in sugar cane (*Saccharum officinarum*). The most important defense response of the plant is the increased production of high-molecular mass and mid-molecular mass glycoproteins (HMMG and MMMG). It has been proposed that arginase activity contained in HMMG glycoproteins avoids teliospore germination in the early stages of infection. Thus, resistance to smut of Mayarí 55–14 sugarcane cultivar has been associated to the accumulation of free or conjugated polyamines in sugarcane tissues. Arginase is the first enzyme involved in polyamine biosynthesis. It catalyzes the hydrolysis of arginine to L-ornithine. From this latter amino acid, plants and fungi are able to synthesize putrescine, spermidine and spermine, one after another. Sugar cane arginase competes with the same enzyme synthesized by teliospores, which, in contrast, has a positive effect on germination, as a false quorum signal.
Relation between polyamines and damage to teliospores is reported herein. Putrescine causes a significant disruption of teliospore germination. Moreover, after incubation of cells with high concentrations of the diamine, fungal spores show alterations in nuclei, which appear decondensed and disorganized in presence of the diamine. Interestingly, most of the treated cells appear as protoplasts which indicates that the diamine could trigger a signalling cascade that involves the rupture of teliospore wall by means of hydrolytic enzymes activities. Tubulin polymerization assays have confirmed that putrescine avoids microtubule stabilization, thereby committing germination. However, low concentrations of spermidine, a later product in the pathway started by arginase activity, significantly favours teliospore germination and tubulin polymerization *in vitro*. Additionally, non-remarkable alterations of nuclei have been found in cells after contact with spermidine.
Thus, a role of spermidine in fungal development is proposed, whereas an accumulation of putrescine, as a result of sugar cane arginase activity increasing during defence response, could become negative for the pathogen.

Cooperation through competition - Dynamics and microeconomics of a nutrient trade system in arbuscular mycorrhizal symbiosis

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In arbuscular mycorrhizal (AM) symbiosis, fungi and plants exchange nutrients (sugars and phosphate, for instance) for reciprocal benefit. Until now it is not clear how this nutrient exchange system works. We used computational cell biology to simulate the dynamics of a network of proton pumps and proton-coupled transporters that are upregulated during AM formation and show that it can function as a nutrient trade system. By applying basic principles of microeconomics, we link the biophysics of transmembrane nutrient transport with the ecology of organismic interactions and straightforwardly explain macroscopic scenarios of the relations between plant and AM fungus. The new insights obtained in this study allow drawing far reaching hypotheses about the mechanism and the regulation of nutrient exchange and propose that the 'cooperation' between plant and fungus can be in fact the result of a competition between both for the same resources in the tiny periarbuscular space.

The study of Physcomitrella patens and Pseudomonas interactions

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Studies of molecular mechanisms of plant's immunity and the origin of phytopathogenic bacteria's parasitism are topical concepts to explore. Mosses are plants which closely related to the first organisms colonized the land more than 450 million years ago. The moss *Physcomitrella patens* is a new model organism for study of interactions between plants and phytopathogens.
The interactions between necrotrophic pathogens such as *Botrytis*, *Pythium*, *Pectobacterium* and moss *P. patens* were previously reported. However, reports about the ability of specialized bacterial pathogens to colonize *P. patens* are absent. So, study of interactions of *P. patens* and specialized *Pseudomonas* plant's pathogenic bacteria is an actual task.
For phenotypic and cytological analyses *P. patens* was cultivated on Knop's medium and inoculated with suspensions of *P. fluorescens*, *P. syringae* and *P. viridiflava* (OD 0.4). Pathogenesis was observed during the month. All inoculated moss colonies became yellow and showed decrease in size in comparison with control. *P. viridiflava* had the most significant impact on moss gametophore and caused yellowing in five-day post inoculation. In addition, significant decrease in size and number of chloroplasts comparing with non-inoculated cells was observed. In ten days after moss inoculation by *P. viridiflava* gametophore's cells of *P. patens* were died.
After two and five days post inoculation *Pseudomonas* inoculated gametophores were washed off and grinded to determine number of bacteria colonies in moss. These suspensions were serially diluted and spread evenly on Petri dishes. As a result, it was shown a statistically significant increase in number of bacterial colonies of *P. fluorescens*, *P. syringae* and *P. viridiflava.*
For metabolome profiling of inoculated *P. patens*, after two and five days post inoculation metabolites were isolated and identified by gas chromatography–mass spectrometry (GC-MS). We found that *Pseudomonas* bacteria induced the accumulation of compounds required for bacterial growth and involved in plant's systemic resistance.
Thus, the obtained data confirmed that *P. fluorescens*, *P. syringae* and *P. viridiflava* are the pathogens of *P. patens*.
The reported study was funded by grant of President of Russian Federation MK -7138.2015.4 and by RFBR according to the research project №13-04-40104.

Peptide elicitors GmPep890 and GmPep914 increase the plants resistance to oxidative stress

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Plant resistance to stresses relies on a number of signaling systems that provide the perception and transmission of extracellular signals into the cell's genome. Defense responses are induced not only by molecules from invading organisms but also by endogenous elicitor molecules generated from plant proteins and recognized as danger signal. The significant progress has been recently achieved in identifying new families of endogenous peptide elicitors. These substances induce and amplify defense responses to invading organisms both locally and systemically. Plant responses include an expression of genes encoding antimicrobial defense proteins, enzymes synthesizing ethylene, jasmonic and salicylic acids, and defense-related secondary metabolites and others.
Peptides GmPep890 and GmPep914 were identified in *Glycine max*. Both peptides consist of eight amino acids, and they were found to induce the expression of typical defense marker genes. Oligopeptides GmPep890 and GmPep914 were synthesized and investigated here for their elicitor activity in the concentration range of 10^{-13}-10^{-9} M using soya and pea plants grown in an aqueous culture. It was shown that that the synthetic peptides GmPep890 and GmPep914 function as elicitors at 10^{-10}-10^{-12} M. One-day treatment of soybean and pea shoots by peptides significantly increased content of soluble phenolic compounds and the antioxidant activities. However, two-days exposure decreased these parameters. This is probably due to the synthesis of lignin and other polymeric phenolic compounds. The synthetic peptides GmPep890 and GmPep914 in concentration 10^{-12} M induced reduction of ROS level and lipid peroxidation products when plant plants were exposed to an oxidative stress ($CuCl_2$/L-

ascorbic acid). The increase of reduced glutathione level was found after treatment by GmPep890 and GmPep914. GmPep914 also increased peroxidases activity (GmPep890 did not change this chracteristics).

Overall, our data demonstrate that treatment of soybean and pea seedlings with peptides GmPep890 and GmPep914 has an ameliorating effect on plant growth under the influence of oxidative stress. The peptide GmPep914 probably triggers stress signalling events and causes defense responses, leading to general resistance to an oxidative stress.

Plants and *Pectobacteria*: to interact means to be modified

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Plant/pathogen systems represent natural biological systems that passed through the evolutionary history. Their formation is not limited to confrontation between defense and attack strategies. Despite this, pathogens are always considered as negative factors only, although they may play important role in ecology of the host. Therefore, pathogen-induced reactions of plants are not restricted to defenses and may include responses necessary for host/pathogen integration resulting in the formation of supra-organismal system. The interaction between the host and pathogen within natural ecosystems usually proceeds asymptomatically and the development of acute pathological process is likely to be an exception related to destabilization of the system due to the particular physiological processes in the host and/or pathogen. Therefore, having insights into the principles of pathosystem development and the reasons of physiological resistance/susceptibility of the plants may provide basis for the designing novel approaches to control the diseases.

The interaction of plants with *Pectobacteria* (one of most harmful phytopathogens) is considered as being restricted to activation of the "brute force" of these bacteria that is related to the synthesis of large amount of extracellular enzymes that degrade plant cell wall. Therefore, most of the investigations on *Pectobacteria* are dedicated to the mechanisms of production of these virulence factors in model cultures or during acute stage of infection in plants. However, there are several prerequisites to consider *Pectobacteria's* "brute force" as not the only way of interaction with the host: 1) the presence of genes that are typical of biotrophic pathogens, and 2) observations that demonstrate the ability of plants and *Pectobacteria* to coexist for a long time without developing the disease symptoms.

Since the events occurring in *Pectobacterium*-infected plants during different stages and different types of infection are largely unknown, we have "looked inside" the infected plants using a variety of complementary methods in order to obtain complex characteristics of different scenarios of plant/*Pectobacterium* pathosystem formation.

Our results show that different compartments of the host-plant colonized by bacteria may form the peculiar signaling background that drives the behavior of microbes resulting in *in planta* dissociation of microbial population. Herewith, various sub-populations display functional specialization. The colonization of the plant may be coupled with a kind of susceptible response that provides conditioning of the particular plant compartment (primary xylem vessels). This response includes site-specific formation of reactive oxygen species that are likely involved in non-enzymatic digestion of some cell wall polysaccharides, including rhamnogalacturonan I (RG I). Herewith, fragments of RG I provide the formation of a gel, which serves as a matrix of complex structures composed of *Pectobacterium* cells – bacterial emboli that were firstly described in our study to be formed in primary xylem vessels of infected plants. These structures are discussing to play a significant role in the formation and development of pathosystem. Additionally, we have revealed that the strategy of interaction is reflected in the peculiarities of plant hormonal system functioning. Our observations were strengthened by the whole-transcriptome analysis using NGS-technologies of both plants and pathogens during their interactions. Taken together, our investigation shows that even those phytopathogens that possess the "brute force" can "feel" their host, apply "gentle" strategy of interaction and induce specific physiological changes of the plants that condition them as an environment of the pathogen.

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Plant-*Pectobacteria* interaction in terms of abscisic acid signalling

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Abscisic acid (ABA) is a plant hormone that regulates responses to the abiotic stressors, water status and ontogenetic processes. Herewith, ABA was also shown to determine several aspects of plant-microbe interactions. However, the possibility of this plant hormone to regulate plant responses to biotic stressors remains very ambiguous. The pathogenesis induced by representatives of Soft-Rot *Enterobacteriaceae* (SRE), including *Pectobacterium atrosepticum* (*Pba*) depends on physiological state of the host-plant including water and ontogenetic status. However, the role of ABA in plant-SRE interactions is unknown. The aim of the present study was to explore the possible roles of ABA in plant-*Pba* interactions. Gene expression analysis was used to monitor ABA-pathway in infected tobacco plants and to check the possibility of ABA perception by *Pba* within the frameworks of plant-pathogen cross-talk. ABA biosynthesis inhibitor application was used in order to asses the impact of ABA on the infection process development.

As ABA-dependent marker genes we have chosen a group of genes that encode ABA biosynthetic enzymes, proteins involved in ABA-signal transduction, and ABA-responsive genes. Previously demonstrated ABA-depended expression and the presence of ABA-responsive cis-elements (ABRE) in the promoters were the defining parameters for gene selection. As a model, we have chosen the inoculated and lower uninoculated plant leaves in order to monitor both the local and systemic transcriptional responses. The expression of selected marker genes was down-regulated during *Pba*-induced infection, pointing to the local and systemic repression of ABA-signaling in the host plant.

The impact of ABA level in plants on pathological system formation was investigated using ABA biosynthesis inhibitor – fluridon. Potato plants were pretreated with fluridon or water, respectively, one day prior to *Pba* inoculation. Fluridon pretreated plants have demonstrated the high susceptibility to *Pba* and exhibited extensive lesions. This indicates that ABA-dependent responses play a positive role in plant resistance to *Pba*.

The responsiveness of *Pba* cells to ABA was analyzed using *in vitro* model by means of classical approaches and Next-Generation Sequencing (NGS). *Pba* cells were cultured in minimal media containing pectin as a carbon source in the presence or absence of ABA. In order to assess if ABA may determine the behavior of *Pba*, several physiological features of this microorganism were monitored. ABA was shown to repress the production of extracellular pectate lyases - one of the main virulence factors of these bacteria; herewith no effect was detected for growth characteristics, pointing to the signaling role of this phytohormone in determination of *Pba* behavior. Mock- and ABA-pretreated *Pba* cultures were further processed for cDNA library preparation in order to perform transciptome analysis using high-throughput sequencing. Several *Pba* virulence-related genes, including those that encode effector proteins of type III secretion system, pectate lyases, regulatory and transport proteins were shown to be ABA-repressed. This is in agreement with the negative effect of ABA on the production of extracellular plant cell wall degrading enzymes by *Pba* and increased susceptibility of plants pretreated with ABA biosynthesis inhibitor to this pathogen.

Thus, our data show ABA-mediated suppression of *Pba* virulence; in turn, the progression of infection is related to the repression of ABA-pathway in the host-plant that is in agreement with the increased susceptibility of infected plants pretreated with ABA synthesis inhibitor. The obtained results are in accordance with the well-known observation: *Pba*-induced disease symptoms are not expressed under low humidity when the concentration of ABA is elevated in plants and symptoms development is usually associated with wet conditions of the environment.

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Some aspects of *Bacillus thuringiensis* entomopathogenic strains influence on pea plants growing activity

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The fundamental investigations of the bacterian Bacillus thuringiensis plants growing stimulative and antistress properties are promising for maximizing crops productivity. There is only limited information available about the treatment of juvenile plants of some crops with *B. thuringiensis* that had a positive effect on seed germination, intensity of shoot growth and increase chlorophyll content in seedlings. However, this problem is actual, that's why it highly needs in the detailed study.
The research is concerned with studying the possible effect of plants growth activity of spore culture of entomopathogenic *B. thuringiensis* strains on the pea plants.
The entomopathogenic strains of *B. thuringiensis* 787 (var. *shandongiensis*) and 0293 (var. *kurstaki*) from the collection of Scientific Research Institute of Agriculture of Crimea was used as a material of research. The action of the studied strains was compared with the effect of strain *B. thuringiensis* Z-52 (var. *kurstaki*). The plants growing action for this referent strain is available for plants of bean and cucumber (Simonova et al., 2008). The distilled water was used as a control. All studies were carried out on the pea Oplot variety.
The germinate vigor and germination of pea seeds witch was treated with the spore cultures of *B. thuringiensis* strains 787, 926 and 0293 ranged from 80,0 to 95,0%. The highest germinate vigor and germination was observed when using the culture of strain *B. thuringiensis* 0293 (95,0%). The increase of germinate vigor was on 22,5 %, and increase of germination was on 2,7% greater compared to the reference strain *B. thuringiensis* Z-52.
Studying the biomorphometric indicators it was identified, that the strain 787 increases the length and weight of the seedling root at 24,2% and 7,9% relative to the control and at 33,2% and 18,1% compared with reference strain Z-52. At the same time, the strains 0293 and 926 didn't show the significant effect on the studied subject.
Furthermore, a study found that treatment with strains 0293 and 926 reduces shoot length of 37,3% and 34,5%, respectively to the control. In the variant with the strain 787 significant changes pea shoot length were observed.
Amylase activity in pea seedlings treated by the *B. thuringiensis* 787 and 926 strains cultures were in 2,17 and 2,72 times higher than the control and in 1,6 and 2,1 times higher than under the reference strain Z-52. The strain 0293 culture didn't affect significantly on the studied parameters.
In water culture conditions (35-days old pea plants) it was showed that 787 strain also has plants growing activity. It was affected in intensification of transpiration by 32,3% to control, decreasing of suction force by 66,6% and increasing the content of green pigments by 55,1%. Comparing with the referent strain Z-52 the intensification of transpiration differed insignificantly, decreasing of suction force was by 42,1% and increasing the content of chlorophyll was by 13,4%.
The action of the strain 0293 was not so significant. But it was observed decreasing of transpiration intensity as compared not only with the reference strain (44,4%) but also the control (21,7%). And it was noted a decrease of suction force by 47,3% compared to Z-52 strain culture and by 69,6%, relative to control. By the action of 0293 strain culture, increasing in chlorophyll content was less find. Compared with the Z-52 strain it was by 5,9% and with the control – by 28,5%.
Thus, it was found, that according to such indicators as biomorphometry and amylolitic activity of seedlings, intensity of transpiration, sucking force of cells and chlorophyll content the bacteria of strain *B. thuringiensis* 787 have the growing activity in pea plants. Taking these facts and the entomopathogenic properties into consideration, we can make a conclusion, that this bacterium is more promising for use in agricultural production.

Bacillus subtilis 10-4 induces salicylic acid accumalation and prevents development of oxidative and osmotic stress in wheat seedlings under salinity

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Salinity is a strong damaging factor limits growth and productivity of plants mainly by develops oxidative stress and water regime disturbance. Earlier we found that *Bacillus subtilis (B. subtilis)* 10-4 (10^5 CFU/ml) exerts protective effect on wheat (*Triticum aestivum* L.) seedlings growth under salinity (2% NaCl). In this work we analyzed the effect of seeds pretreatment with *B. subtilis* 10-4 on the dynamic of an important osmoprotectant - proline and malondialdehyde (MDA) (the final product of lipid peroxidation) concentrations in wheat seedlings under salinity. It was found that pretreated with *B. subtilis* 10-4 decreased stress-induced proline accumulation level in seedlings. Apparently, this is a consequence manifestation by *B. subtilis* preadaptation effect on plants to stresses leading to water deficit. Also, the obtained data showed exposure to salt stress caused essential increase of MDA in plants. At the same time *B. subtilis* 10-4 reduced the level of stress-induced lipid peroxidation, which in turn, says about weakening of oxidative stress. We can assume that the protective effect of these bacteria related to the modulation activity of oxidative enzymes under their influence, so they can control the level of hydrogen peroxide, which induces lipid peroxidation, the end product of which is MDA. Meanwhile activating of *B. subtilis* induced defense reactions may be connected with their ability to increase content of salicylic acid (SA) that plays important role in induction of systemic resistance in plants. Thus, the obtained data indicate that pretreatment of seeds with *B. subtilis* 10-4 leads to accumulation of SA in seedlings and reduces the level of stress-induced accumulation of proline and MDA that makes a significant contribution to realization by *B. subtilis* protective effect on wheat plants under saline conditions.

Implication of the cytoskeleton in the displacement, germination and infectivity of smut teliospores, a sugarcane pathogen

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Smut is a major disease of sugarcane caused by Sporisorium scitamineum. A primary response of sugarcane plants to the infection seems to be the production of several glycoproteins, defined as mid-molecular mass (MMMG) or high molecular mass (HMMG) macromolecules. The concentration of these glycoproteins clearly increases after inoculation of sugarcane plants with smut teliospores. Teliospore germination in the presence of both MMMG and HMMG decreased about 50% following 5 h of teliospore contact with glycoproteins. This may be related to the ability of glycoproteins to produce cytoagglutination. Moreover, a unique application of salicylic acid, naturally produced by sugarcane stalks after experimental fungal infection, enhanced the production of both glycoprotein pools.
Six different proteins have been identified in the pool of HMMG: arginase, β-1,4-glucanase, β-1,3-glucanase, chitinase, a dirigent protein and an unknown peptide that acts as chemotactic agent. Arginase from healthy plants binds to cell wall teliospores and it is completely desorpted by sucrose, but only 50% of arginase activity from inoculated plants is desorpted by the disaccharide. This arginase induces cytoagglutination of smut teliospores but impedes germination.
The chemotactic movement of teliospores, directed to facilitate their entry into the plant tissues and to achieve cytoagglutination, is strongly inhibited by phalloidin and latrunculin A, which implies that polymerization and depolymerization cycles of F-actin of teliospores are required to move, and by blebbistatin, which implies a contractile protein similar to a myosin II, responsible for the contraction–relaxation of the cytoskeleton. In addition, microtubules seem to be also involved in the process since nocodazole inhibits chemotactic displacement.

Dirigent protein seems to be related to the accumulation of hydroxycinnamic acids in resistant plant tissues after smut inoculation, following an increase in phenylalanine ammonia-lyase and cinnamoyl alcohol dehydrogenase activities. However, sensitive cultivars, such as Barbados 42231 showed low dirigent protein activity as well as low phenylalanine ammonia-lyase activity as a consequence, this last, of the accumulation of caffeic acid that produced feedback inhibition of the enzyme.

The role of polyamines in fungal development during Sporisorium scitamineum-sugar cane interaction

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Sporisorium scitamineum is a filamentous fungus that causes smut disease in sugar cane (Saccharum officinarum). The most important defense response of the plant is the increased production of high-molecular mass and mid-molecular mass glycoproteins (HMMG and MMMG). It has been proposed that arginase activity contained in HMMG glycoproteins avoids teliospore germination in the early stages of infection. Thus, resistance to smut of Mayarí 55–14 sugarcane cultivar has been associated to the accumulation of free or conjugated polyamines in sugarcane tissues. Arginase is the first enzyme involved in polyamine biosynthesis. It catalyzes the hydrolysis of arginine to L-ornithine. From this latter amino acid, plants and fungi are able to synthesize putrescine, spermidine and spermine, one after another. Sugar cane arginase competes with the same enzyme synthesized by teliospores, which, in contrast, has a positive effect on germination, as a false quorum signal.
Relation between polyamines and damage to teliospores is reported herein. Putrescine causes a significant disruption of teliospore germination. Moreover, after incubation of cells with high concentrations of the diamine, fungal spores show alterations in nuclei, which appear decondensed and disorganized in presence of the diamine. Interestingly, most of the treated cells appear as protoplasts which indicates that the diamine could trigger a signalling cascade that involves the rupture of teliospore wall by means of hydrolytic enzymes activities. Tubulin polymerization assays have confirmed that putrescine avoids microtubule stabilization, thereby committing germination. However, low concentrations of spermidine, a later product in the pathway started by arginase activity, significantly favours teliospore germination and tubulin polymerization in vitro. Additionally, non-remarkable alterations of nuclei have been found in cells after contact with spermidine.
Thus, a role of spermidine in fungal development is proposed, whereas an accumulation of putrescine, as a result of sugar cane arginase activity increasing during defence response, could become negative for the pathogen.

The role of hormonal signaling in the resistance of wheat plant infected with *Septoria nodorum* Berk.

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The development of defensive reactions under the influence of biotic stress factors depends largely on the ability of plants to produce a variety of plant hormones and signaling molecules play a key role in the induction of systemic resistance against plant pathogens. Salicylic (SA), jasmonic (JA) acids and ethylene play an important role in immunity plants. However, it shown that the immune system of plants is regulated by phytohormones such as cytokinins (CK) and abscisic acid (ABA). Influence of signaling pathways is still not fully explored, and information about the role of the CK and ABA in the pathogenesis contradictory.
We have studied the effect of the SA, the ethylene, the CK and ABA on the development of protective reactions in two contrasting varieties of wheat plants (*Triticum aestivum* L.) infected by the hemibiotrophic fungus *Septoria nodorum* Berk. In the initial period of infection with resistant varieties Omskaya 35 (Om35) was found intense generation of hydrogen peroxide (H_2O_2), a high activity of peroxidase (PO) and a decrease in the activity of catalase (CAT). In the susceptible varieties Kazakhstanskaya 10 (Kaz10) low PO activity and increased CAT activity was the reason for the weak generation of H_2O_2 and the rapid development of the pathogen in the leaves. Pre-sowing treatment with

SA and treating leaves with 1-methylcyclopropene (1-MCP) (ethylene inhibitor binding to its receptors) increased resistant of both varieties of wheat plant to *S. nodorum*, which was manifested in a decrease in affection zones by increasing the generation of H_2O_2, increased activity PO and inhibition of CAT activity. These are confirmed by our data about increase of damage zones and reduce activity PO in the infected plants of both varieties of wheat treated ascorbate (ASP). Interestingly, treating leaves with ethephone (2-chloroethylphosphonic acid) (ET) resulted in a decline in wheat resistance to infection, manifested in vast zones of damage with chlorosis which is likely to be due to decreased generation of H_2O_2, increased CAT activity and inhibition of PO. In addition, we observed that the infected plants of resistant variety Om35 and plants treated with SA and 1-MCP both varieties accumulated transcripts genes encoding protective proteins (PR-1, PR-2, PR-3, PR-9) which was not detected in the treated ET infected leaves of wheat. Based on these results, we can assume that SA-dependent signaling system induced in the resistance forms of wheat plants infected with *S. nodorum* by the generation of ROS and the antagonistic effect of ethylene-dependent signaling pathway.

In this regard, interest our data to increase endogenous content CK and reducing endogenous content of ABA in the case of increasing the resistance of plants to pathogen (Om35, treatment SA and 1-MCP) and, on the contrary, accumulation of ABA and no significant increase in the CK content in case of low sustainability (Kaz10, treatment ET). Treatment of wheat plants with CK resulted in reducing lesions on the leaves, and ABA treatment, on the contrary, such an increase. The protective effect of CK on the infected plants was significantly reduced in the leaves treating of ASP, which is likely to be due to reduced PO activity. Histochemical analysis of phytohormones distribution showed that in the case sensitivity to pathogen of the CK and ABA localized mainly in developing fungal structures, whereas most plant cells have been deprived of phytohormones, particularly CK. In the case of the resistance to pathogen of the CK were located in the plant cells, and fungal structures are not developed. This involves the activation of the signal system of the CK, leading to the start of defense reactions in plants.

Therefore, our results suggest that resistance of wheat plant to *S. nodorum* regulated due to antagonism of signaling pathways of salicylic acid and ethylene, with the participation of cytokinins.

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Local and systemic signaling in plant/microbe interaction mediated by a novel chemical mediator

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Plants are exposed to numerous threats. They are perceived by microbe- / damage-associated molecular patterns or other chemical mediators which initiate appropriate local and systemic responses in the stress-exposed plant. We identified a novel chemical mediator which induces rapid cytoplasmic calcium elevation in roots and shoots. Downstream of calcium elevation in the roots, the production of reactive oxygen species as well as the activation of defense responses can be detected locally in roots as well as systemically in leaves. Mutant analyses in Arabidopsis demonstrate that calcium elevation is necessary for the downstream responses. I will discuss the role of the chemical mediator for plant performance in different environments and compare the compound with known pathogen-associated molecular patterns.

Evolution of actinorhizal symbioses: root nodules induced by members of the basal group of *Frankia* strains

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Two types of root nodule symbioses exist, legume/rhizobia and actinorhizal symbioses, and both go back to a common predisposition which was acquired by the progenitor of the Fabid clade. In actinorhizal symbioses, nitrogen-fixing soil actinobacteria of the genus *Frankia* induce the formation of root nodules on a diverse group of dicotyledonous plants from eight families from three different orders, Fagales, Cucurbitales and Rosales. Phylogenetically, frankiae can be divided in four clusters, three of which (clusters I, II and III) represent symbiotic strains. Cluster II is the ancestral one; these strains show strong genome reduction and with one exception could never be cultured. The strains of cluster II can enter symbioses with host plants from four different families present on four different continents, actinorhizal Rosaceae and and one rhamnaceous genus, *Ceanothus*, from the order Rosales and Coriariaceae and Datiscaceae from the order Cucurbitales.
Actinorhizal nodules from different host plant genera show great diversity regarding infection pathways, anatomy, *Frankia* differentiation in infected cells and nodule metabolism; in particular, strong differences exist between host plants from different orders.
In order to understand the evolution of root nodule symbioses, we are working on the interactions of Frankia cluster II strains. We compared the genomes of an Asian and an American strain. On the plant side, we compared the plant and bacterial transcriptomes of two host plants of cluster II strains, *Datisca glomerata* (Cucurbitales) and *Ceanothus thyrsiflorus* (Rosales). We also analysed infection pathway and differentiation of infected cells in *D. glomerata* as a model system for actinorhizal Cucurbitales.

Defence sugarcane glycoproteins cause microtubular disorganization and defects in structure and migration of nuclei of *Sporisorium scitamineum* teliospores

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Resistant sugar cane plants naturally produce defence glycoproteins that accumulate in the parenchymatous cells of stalks and prevent the infection by the filamentous fungi *Sporisorium scitamineum*. These glycoproteins, which have been defined as macromolecules of medium and high molecular weight (MMMG, Mid-Molecular Mass Glycoproteins and HMMG, High-Molecular Mass Glycoproteins), induce chemotaxis, homotypic adhesion and inhibit teliospore germination.
The inhibitory effect on germination caused by sugar cane glycoproteins seems to be related to microtubular disorganization in fungal cells. During germination, nuclear migration occurs in parallel to a strong polarization of microtubules (MTs) at the basis of the hyphae. Tubulin polarization seems to be required for pathogen development since 8 μg mL^{-1} nocodazole reduces fungal germination in a 50 % after 15 hours of treatment. Interestingly, after contacting with sugar cane HMMG glycoproteins microtubule polarization in fungal cells does not take place, either. Microtubule immunolabeling images reveal a decrease of approximately 70% in the number of polarized cells in presence of glycoproteins produced by resistant varieties of sugar cane. Treated cells exhibit a homogeneous fluorescent immunolabeling whereas control teliospores show an evident crescent-shaped fluorescence distribution as a result of polarized microtubules. Similarities with nocodazole effects indicate that microtubules disorganization must be involved in a failed germination. Moreover, as a consequence of a non-correct arrangement of microtubules, teliospores exhibit nuclear and microtubular alterations after incubation with HMMG glycoproteins. Nuclei, which appear decondensed and fragmented, cannot correctly migrate through the growing hyphae and germination fails.

Arginase activity contained in defence glycoproteins is already described for preventing fungal germination. Now, its enzymatically active form is presented as a link between the defensive capacity of glycoproteins and the MT disorganization in fungal cells. Active arginase is constitutively produced by healthy resistant plants whereas it is not detected in the juice from susceptible varieties. That is why MT depolarization, nuclear disorganization as well as germination of teliospores are not significantly affected by glycoproteins from non-resistant plants. Susceptible to smut disease plants try to increase the arginase activity in juice after detecting the presence of the pathogen but it is "too late" for an effective response and plants are not able to defend themselves.

Defensive role of lignans in the response of sugar cane against *Sporisorium scitamineum* infection

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Lignin is a phenolic polymer that provides an additional structural resistance to the plant cell wall as well as an increase of the hydrophobicity and resistance to the entry of the pathogen. The production of dimers and trimers from monolignols requires of guidance proteins (DIR proteins), a family of proteins, exclusive of plants, involved in the production of lignins and in responses to the invasion by pathogenic organisms and abiotic stresses. These proteins are responsible for the stereo-selective coupling of monolignol radicals to produce lignans or, alternatively, lignin.
Smut, caused by the fungus *Sporisorium scitamineum*, is an important disease of sugar cane in many sugar cane-growing regions of the world. *SofDIR16* mRNA expression in both sugar cane meristem and leave sections of Mayarí, a sugar cane resistant variety to smut disease, is higher than in Barbados, a susceptible variety. Interestingly, *SofDIR16* expression increases in the susceptible variety whereas it is almost nullified in the resistant variety after inoculation with smut sporidia. It has been proposed that lignans are related to the defense response of Mayarí variety to smut since the decrease of DIR expression in the resistant variety could be directly related to lignan accumulation.
Lignan content in meristems of sugar cane plants has been analyzed by capillary zone electrophoresis (CZE). Pinoresinol seems to play an important role in sugar cane defense. Pinoresinol level in meristems seems to be constitutively higher in the resistant variety than in the susceptible one. Moreover, pinoresinol concentration increases in Mayarí plants after sporidia inoculation whereas it decreases in non-resistant plants. Pinoresinol is not accumulated in meristems after water injection which means that lignan synthesis is a very specific response triggered by the pathogen in resistant plants, but not by wounding.
When smut sporidia are maintained in the presence of commercial pinoresinol, about 50 % of it, disappears in the medium after 48 h. These sporidia presented an altered morphology as a consequence of the incubation with the lignan. The uptake of pinoresinol by the pathogen could be responsible for the morphological alterations detected in fungal cells. In the same way, nuclear alterations have been observed in fungal cells after contact with commercial lignans, which strengthens the idea of their cytotoxic effect.

Plant-microbe signal exchange leads to the highly specific symbiosis development

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Symbiotic receptor kinases play a crucial role in development of legume plants under cooperation with soil microorganisms. In particular, receptor kinases determine the specificity of interactions with nodule bacteria, take part in signaling during symbiosis development and regulate the intensity of microbial colonization. Investigation of structure and functions of these kinases can help in understanding how plant reacts to environmental conditions, and also can be useful for breeding in conditions of sustainable agriculture.

Specificity of interactions between legume plants and nodule bacteria is based on ligand-receptor interactions, during which the bacterial signal molecules are perceived by plant receptor kinases. Lipochitooligosaccharide signals emanating from rhizobia, known as Nod factors (NFs), trigger a complex of specific responses in the epidermis, pericycle and root cortex of the legume plants, thereby providing the basis for subsequent bacterial entry and organogenesis of root nodules. For many years it has been predicted that legume plants perceive NFs by means of high affinity receptors, triggering signal transduction pathway. Among the evidence is the fact that minor changes in NF structure can change rhizobial host range. Despite some candidate NF receptor genes enconding LysM-receptor-like kinases (LysM-RLKs) have been identified in pea *Pisum sativum*, the underlying mechanisms by which binding of ligand elicits signaling responses remains unclear.

The focus of our research is to unravel these highly specific mechanisms by which rhizobial NFs produced by the symbionts are perceived by the legume plants. To address this aim some new putative LysM-RLKs (K1 and LykX) have been found and characterised in pea. TILLING analysis has allowed to select mutants in these genes and to characterise their phenotypes. Also the natural polymorphism of these genes was examined in 99 pea genotypes that represent virtually all the diversity within the genus *Pisum*. As a result, it was demonstrated that the allelic state of *LykX* gene is associated with the ability of plants to form symbiosis with wide spectrum of bacterial strains.

The possible role of new receptors will be discussed. In particular, we propose the model for perception the NF molecule by various receptor complexes. The applied aspects of the study will also be discussed, for example, the enzymatic synthesis of chitooligosaccharides and induction of plant defence genes after application of these molecules.

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Nod factor perception and signal transduction during endosymbiotic interactions of *Medicago truncatula*

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The legume *Medicago truncatula* maintains endosymbiotic interactions under phosphate and nitrate limiting conditions with respectively mycorrhizal fungi and rhizobia. Both microsymbionts produce lipochitooligosaccharides (LCOs) which are perceived by plant receptors with extracellular LysM domains and an intracellular kinase domain (LysM-RLKs). Mycorrhizal fungi in addition secrete short chain chitooligosaccharides (COs) which also function as signal molecules. Three LysM-RLKs from *M. truncatula*, LYR3, LYK3 and NFP, were studied in detail in our group. LYR3 is a high affinity binding protein for LCOs but not for COs. Swapping experiments with LysM domains from different plant species identified the crucial role of the third LysM motif in LCO binding. LYR3 from two *Lupinus* species which do not form the mycorrhizal symbiosis are deficient in high affinity binding to LCOs. LYR3 and NFP lack an active kinase domain and LYR3 is phosphorylated by the active kinase domain of LYK3. FRET experiments showed that LYR3 and LYK3 interact at the plasmamembrane and this interaction is inhibited or disrupted by addition of LCOs. Co-expression of NFP and LYK3 in tobacco leaves provokes a cell death response that is attenuated in the presence of LYR3. Thus LYR3 may play a role in regulating the functional interaction of NFP and LYK3. Low level expression of these 3 proteins hampers their visualization in roots of *M. truncatula*. Ongoing experiments to increase the detection sensitivity and thus study the distribution and regulation of these proteins during endosymbiotic interactions will be presented.

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False quorum-signal establishment: sugar cane and *Sporisorium scitamineum* arginases competition

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Smut is a major disease of sugarcane caused by the filamentous fungus *Sporisorium scitamineum* (Syd.). Resistant to smut sugar cane cultivars produce a pool of glycoproteins and one of them develops arginase activity. Sugar cane arginase is responsible for agglutination as well as for teliospore germination inhibition. Surprisingly, teliospores of *S. scitamineum* also secrete its own arginase. However, fungal arginase activates a signal transduction cascade that accelerates teliospore germination when it binds to fungal cell wall. Competition assays between sugar cane and fungal arginase are reported. The affinity of active fungal arginase (AFA) for its ligand shows to be higher than that found for sugar cane arginase (SCA). In fact, AFA, that enhances significantly teliospore germination, removes previously-bound SCA whereas the inverse process is practically negligible.

Moreover, enzymatic activity of arginase is required for binding to teliospore cell wall and to activate germination in cells. Binding of inactivated fungal arginase (IFA) to teliospore wall is very ineffective and it is easily reversed by AFA. In the same way, arginase activity is necessary to stimulate germination, maybe by means of putrescine synthesis. The addition of putrescine to germinating teliospores does not significantly alter the rate of germination for concentrations from 0 to 1.0 mM although it is slightly inhibited by 5.0 mM diamine. However, the frequency of sporidia formation is considerably enhanced by 0.1 mM putrescine indicating that putrescine, product of arginase activity, increases the potential infectivity of the pathogen. A transient and controlled depolarization of cytoskeleton induced by low concentrations of putrescine could stimulate sporidia liberation but an elevated putrescine production as a consequence of SCA could not be regulated by teliospores and the effect would become negative.

Thus, if SCA binds to the teliospore wall before AFA production, the germination initiated by the fungal enzyme cannot take place. The binding of early produced SCA will avoid germination whereas fungal arginase still has not been secreted to activate the germination signaling cascade. Agglutination not linked to germination is then the result of a false quorum signal triggered by sugar cane arginase.

SESSION 9

Response to Radiation and Gravity

Effect of microgravity conditions on germination of *Brassica napus* seeds

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The strength and direction of gravitational field is homogenous in terrestrial environment. This feature makes it the most important factor defining the polarity of plant growth and development, and underlying the phenomenon of gravitropism, i.e. polarized growth on gravity field. Indeed, plants are able to distinguish the vector of gravity force and adjust the position of root and shoot in the way optimal to access light, water and mineral salts. However, the mechanisms of growth polarity maintenance are still not completely understood. Moreover, the impact of individual proteins in establishment of growth polarity by seed germination is still to be characterized. To address these mechanisms, we characterize here the changes in *B. napus* seedling proteome in response to the loss of gravity vector by application of microgravity conditions by rotation in a 2D clinostat. All experiments (n = 3) were performed twice, and were accompanied with comprehensive biochemical and physiological characterization of plant state. Our analytical strategy relied on LC-based bottom-up proteomic approach employing high-resolution data-dependent acquisition experiments (Thermo Fisher Scientific Orbitrap Fusion Tribrid mass spectrometer).
Although biochemical analyses revealed no essential oxidative stress, the application of microgravity conditions triggered significant changes in abundances of individual proteins. The overall numbers of up- and down-regulated proteins (approximately 1.5-3.3-fold changes, ANOVA test: $p \leq 0.05$) found in at least one experiment, were 160 and 100, respectively. An essential part of affected proteins were involved in energy metabolism and ribosome biogenesis. The patterns of the proteins obtained after one day of clinostat rotation just partially overlapped with that observed one day later. Thus, glyceraldehyde-3-phosphate dehydrogenase (cytoplasmic and chloroplast isoforms), phosphoenolpyruvate carboxykinase, 6-phosphogluconate dehydrogenase and cytoplasmic ribosomal protein L3A were up-regulated during the both observation days. These results indicate higher degree of energy metabolism activation and protein biosynthesis level in the absence of constant vector of gravity field.

Unexpected turns in gravitropic curvatures

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Gravitropic reaction – the process of plant organ orientation in accordance with gravity vector direction – belongs to the most important factors in plant organism development. The widely spread version of gravitropic response, which is accompanied by the formation of stem curvature, is the restoration of stem vertical position after plant inclination. The major mechanism of gravitropism that is discussed for herbal plants is based on the non-uniform elongation of cells located on the opposite stem sides, occurring in the growing zone of an organ. However, gravitropic response may be well-pronounced in

the lower part of developing stem, which has ceased elongation long in advance of plant inclination, as it happens in flax stem. We have analyzed such stem curvature region by various approaches and found the undescribed earlier profound modifications of gelatinous fibres - highly specialized plant cells that are considered to serve as "plant muscles". The observed changes occurred at various combinations of gravitational and mechanical stimuli, irrespective to stretching or compression of the stem side during induction or development of gravitropism, provided it is the pulling side of the stem. Removal of stem apical part before plant inclination didn't affect the changes observed in fibers, same as dynamics of plant vertical position restoration.
We performed large-scale transcriptome profiling of fibers isolated from pulling and opposite sides of gravitropic curvature and compared to fibers of control plants. That helped to highly enrich the understanding of gravitropic response processes specifically in gelatinous fibers. Significant changes in transcript abundance take place for genes encoding proteins of various aspects of cell metabolism, including cell wall enzymes, ion channels, components of hormonal regulation system, etc.
The described profound changes in phloem fibres in the course of gravitropic response raise up the question on the perception and transmission of signal to these cells. At the stage of cell wall thickening, flax fibres constitute isolated symplastic domains as they do not have plasmodesmata, which are destroyed at earlier developmental stage, during intrusive elongation. It means that these cells either perceive the signal themselves or get it through the apoplast. Since fibres are unique in cell length, they have very specific physical parameters "to feel" the stem inclination and may be not only the active motor cells, but also the gravisensing ones. Vertical position restoration by plants with removed apical part indicates that meristem and growing zone are not the only stem regions that percept and transduce the gravitropic signal and perform gravitropic responce.
Phloem fibres with constitutively formed gelatinous cell wall, located in non-elongating parts of herbal plant and involved in gravitropism, may become an important element of study aimed to general understanding of the gravity effects on plant organisms. System is not complicated by growth processes: cell elongation or cambium initial differentiation are not involved in the reaction of primary phloem fibres to gravitational stimulus. All the above makes us to suggest flax phloem fibres as the model system to study the mechanism of gravitropic responce, including signal perception and transduction. Understanding of the phloem gelatinous fiber role may considerably renew the concept of graviresponse and, more generally, of plant organ movement in plants, including the herbaceous species.
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Molecular basis of environmental stresses-induced mutagenesis in *P. patens*

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Owing to their sessile lifestyle, plants are often exposed to extreme environmental conditions leading directly or indirectly to DNA damage. The most cytotoxic DNA lesions are double strand breaks (DSB), in eukaryotes preferentially repaired by non-homologous end joining (NHEJ). In this respect the moss *Physcomitrella patens* is an exception because of its high frequency of homologous recombination (HR) and other unique features, which make it an excellent model plant to study of DNA damage response (DDR). The key player in early DSB response is MRN, thought to be involved in both HR, as well as NHEJ mechanism through ATM signalling. The importance of MRN is manifested by hypersensitivity of *Physcomitrella* lines lacking functional MRN complex to radiomimetic DSB's inducing agent Bleomycin. The combined study of direct DSB repair by single cell gel electrophoresis (comet) assay and of induced mutagenesis in *APT* (adenosine phosphoribosyltransferase) locus in a *Physcomitrella* protonemata culture with 50% of apical cells revealed that repair defect is not the cause of sensitive phenotype, because wild-type *Physcomitrella* and lines with disrupted canonical DSB's repair pathways has identical repair efficiency and rates. The hypersensitivity of mutated lines is rather consequence of effective, but error-prone repair (e.g. NHEJ) leading to induction of mutations that we identified in *APT* locus, nevertheless, which are induced in the whole genome. Relation among identified mutations and observed phenotype will be discussed.

Development of gravitropic response: unusual behavior of flax phloem G-fibres

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Gravitropic response of flax (*Linum usitatissimum* L.) plants is well expressed: several days after the forced inclination for 90° relative to gravity vector direction, plants correct their position in space and return into vertical position. The major input into the restoration of stem position is made by the curvature formed in the stem part that has stopped elongation long before gravistimulation. We applied various approaches of microscopy to analyze the stem curvature region and found the previously uncharacterized significant modifications in primary phloem fibres that have constitutively developed G-layer. In fibres on the pulling stem side, cell portions were widened with formation of "bottlenecks" between them, leading to the "sausage-like" shape of a cell. Lumen diameter in fibre widening increased, while cell wall thickness decreased. Callose was deposited in proximity to "bottlenecks" and sometimes totally occluded their lumen. Structure of fibre cell wall changed considerably, with formation of breaks between G- and S-layers. Thick fibrillar structures that were revealed in fibre cell wall by light microscopy got oblique orientation instead of parallel to the fibre axis one in control plants. Thus, phloem fibres with constitutively formed gelatinous cell wall, located in non-elongating parts of herbal plant, are involved in gravitropism and may become an important element in general understanding of the gravity effects on plants.
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Effect of gamma radiation on root growth in *Arabidopsis thaliana* plants from Chernobyl zone and plants lacking key ion transport and signaling systems

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Introduction. Approximately 80% of radionuclide fallout caused by Chernobyl was received by Belarus. Therefore the effect of radiation on living systems including plants has a special significance for this European country. Plant systems are generally more resistant to gamma irradiation than animals. Moreover, low doses of gamma radiation can stimulate plant growth causing 'radiation hormesis'. However, mechanisms of radiation effects on plant growth at cellular and molecular levels, are largely unknown.
The aim of this study was to characterize growth of different Chernobyl lines and KO mutants of *Arabidopsis thaliana* L. Heynh treated by different doses of exogenous γ-radiation (0.5-30 Gy).
Materials and methods. The growth rate of main root was measured in sterile vertically-grown culture. A number of genotypes were tested including the following: Chernobyl zone plants (collected at effective doses of 0.7 and 5 μSv), knockout mutants of K^+ efflux channel (*gork1-1*), DNA damage response genes (*atm1-1*, *atr1-1* kinases) and histone acetylation systems (*yaf9-a1, yaf9-b2*). Irradiation was carried out during 60 min and then measurement of root length was undertaken during 7 days.
Results. We have found that low doses of γ-radiation (0,5-3 Gy) stimulate WS-0 and Col-0 root growth while high doses inhibit this process. Plants collected in the Chernobyl zone on territories with background radiation of 0,7 μSv have demonstrated extreme sensitivity to low doses. However plants collected in Chernobyl at 5 μSv were similar to control WS. The effect of growth stimulation by low doses was much lower in KO lines of ATM kinases comparing to WS. The knockout of ATR kinases reacted to radiation similar to control WS. Surprisingly, growth stimulation by radiation decreased in KO line of YAF9-B2 histone acetylation control system while the closely related homologue YAF9-A1 demonstrated response closed to WT. Intriguingly, plants lacking outwardly-rectifying potassium channel GORK, demonstrated insensitivity to low doses and did not show a stimulation effect. The inhibition of growth by higher doses of gamma radiation demonstrated significant complexity of effects.

Conclusions. This study showed that low doses of γ-radiation (0.5-3 Gy) stimulate root growth of *Arabidopsis* plants, while high doses (5-30 Gy) inhibit this process. Different ecotypes and genotypes demonstrate specific response to radiation which requires further detailed investigation.
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Closer look on chernobyl area-grown soybean seeds and their adaptation to increased level of ionizing radiation

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Despite of remaining ionizing radiation plants keep growing and reproducing in the radioactive Chernobyl area. However a comprehensive characterization of these mechanisms was missing. Our study reveals that adaptation of investigated soybean plants in this radioactive environment has led to alteration of the developing soybean seed proteome in a specific way that resulted in the production of fertile seeds with low levels of oil and β-conglycinin seed storage proteins. Soybean seeds were harvested from plants grown in non-radioactive (control) and radioactive plots in the Chernobyl area at different stages: four, five, and six weeks after flowering, and at maturity. The abundance of 211 proteins was determined. The results also confirmed previous data indicating that alterations in the proteome include adaptation to heavy metal stress and mobilization of seed storage proteins. The results also suggest that the carbon metabolism in the cytoplasm and plastids has been modified, the activity of the tricarboxylic acid cycle increased and the malonyl-acyl carrier protein condensation decreased during the fatty acid biosynthesis.

Ethylene is involved in the actin cytoskeleton rearrangement during the root gravitropic response of *Arabidopsis thaliana*

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Gravitropism, the directed plant growth with respect to the gravity vector, is regulated by auxin and its polar transport system, several secondary messengers, and by the cytoskeleton. Recently we have shown that the actin cytoskeleton in the root transition zone of *Arabidopsis thaliana* (L.) Heynh was rearranged after gravistimulation (rotation by 90°): the fraction of axially aligned microfilaments decreased and the fraction of oblique and transversally-oriented microfilaments increased. In the present research we have studied the effect of ethylene and inhibitors of its synthesis on actin cytoskeleton rearrangement during the gravitropic response. Application of the ethylene releasing substance ethephon to *A. thaliana* seedlings led to the disassembly of actin microfilaments as well as their broad angle distribution in cells of the root transition zone. This actin rearrangement was escaped by treatment with the ethylene synthesis inhibitor aminoethoxyvinylglycine (AVG). Another negative regulator of ethylene, salicylic acid, was shown to disturb actin microfilament rearrangement as well. We conclude that ethylene is essential for the process of actin cytoskeleton rearrangement in root cortex cells during the gravitropic bending response.
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Genome stability in plants from Chernobyl zone is facilitated by DNA-repair pathways

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Despite of high contamination for almost 30 years, flora in Chernobyl zone continues to flourish, evidencing the adaptation of plants to chronic radiation and heavy metals in the soil. One of the immediate targets of radiation is the genetic material, DNA which can be irreparably damaged. DNA damage activates DNA-damage response (DDR) which mediates radiation-dependent cell cycle arrest and triggers the induction of DNA repair. In plants, DDR pathways must be extremely efficient since plants are sessile organisms and have to accommodate their life to the contaminated environment. However, the genetic basis of the adaptation to the extreme environment is still not fully understood. Such knowledge is of special interests because it provides basis for biotechnology for crop improvement and soil remediation. For our investigations, seeds of *Arabidopsis thaliana* have been collected in contaminated sites of Chernobyl zone. Recently developed micro-phenotyping system has been used for assessment of natural *Arabidopsis* tolerance (root length and nucleus integrity) to radiomimetic and heavy metal impact. A certain degree of variation with regard to their ability to tolerate heavy metal stress and DNA damage by radiomimetic agents has been observed among *Arabidopsis* plants collected from different sites. More tolerant *A. thaliana* lines from Chernobyl zone were selected as experimental models for further QTL analysis. Treatments with radiomimetic revealed increasing expression of genes involved in DDR pathways and cell cycle control. In particular, expression of *ATR* kinase gene and downstream expression of *CycB1;1* was increased after bleomycin and zeocin treatments suggesting role of ATR-dependent pathway in DNA repair and cell cycle control in genome stabilization in Chernobyl plants. We continue investigations on other mechanism of DDR including cell cycle regulation and PCD hallmarks in order to compose the complete picture on the mechanism of plant tolerance to anthropogenically contaminated environment.

Simulated microgravity induces specific alterations in the metabolite profiles of germinated *Brassica oleracea* L. seeds

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Gravitation is one of the most important polarized environmental factor defining the development of plant organisms in space. Every plant can 'estimate' its position with respect to the gravity vector and correct it, when necessary, by means of polarized growth. However, plant organisms at the space stations are growing in the microgravity, which is leading to the altered physiology in comparisons to the plants on the planet.
On the Earth microgravity can be closely simulated using clinorotation or random positioning – rotation of the plant around the horizontal axis (or several axes), which is disorients studied object in the gravity field. Microgravity simulation using the continuous changes of object´s orientation relative to the gravity vector can generate the effects comparable to the effects of true microgravity.
Experiments in space biology have provided a wealth of knowledge pertaining to physiology, genomics and proteomics of plants grown under the microgravity conditions. Nevertheless it should be mentioned that plant growth and development changes under microgravity also associated with the alterations in plant metabolome. We report the metabolic and developmental changes that occur with the germinated *Brassica oleracea* L. seeds under the simulated microgravity.
In this study, we used clinostat with two axes, which is often referred to as 'random positioning machine' or three-dimensional (3-D) clinostat. 3D random rotation could provide a better simulation of the weightlessness conditions as compared to the classical 2D clinostat. Our experiments show that the 3-D

clinorotation leads to the disorientation of root growth with respect to the gravity vector and affect the following development of seedlings, causing the larger number of abnormally developed seeds.
The content of low-molecular-weight substances in excised embryonic axes and cotyledons of *B. oleracea* seeds germinated for 24 h and 48 h under the simulated microgravity has been evaluated using the gas chromatography– mass spectrometry. For every metabolomic profile, we estimated 129 target substances, and 34 of them were unambiguously identified. These compounds included amino acids, organic and fatty acids, tocopherols, phytosterols.
To keep the data assay within the context of multivariate statistics, we used the principal component analysis (PCA). The analysis revealed a significant difference between the metabolomic profiles of excised embryonic axes in 24 h germination group, with the primary role of the amino acids and organic acids. Noticeably, the 1st and 2nd principal components showed 68 % of the explained variance and, according the cross-validation test, 74 % accuracy of identification. The predictive squared correlation coefficient (Q_2) was 61 %. The significant difference between the metabolomic profiles of the cotyledons appeared only in 48 h germination group with explained variance of 64 % and Q_2 equal to 67 %.
Thus, the embryonic axes were sensitive to the disorientation in space already on the seed germination stage. Metabolomes of the cotyledons demonstrated the difference in the stage of seedling development.
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Multiple cell wall-related mechanisms mediate the effect of brassinosteroids on Arabidopsis hypocotyl gravitropism

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Gravitropism can be defined as the directed growth of plant organs with respect to the gravity vector. Plant shoots normally demonstrate negative gravitropism growing upright, in the direction opposite to the gravity vector.
In the present work we studied the role of cell walls in the control of the negative gravitropism of hypocotyls from etiolated *Arabidopsis thaliana* seedlings using brassinosteroid-related treatments as experimental tools. We found that hypocotyls of plants grown on horizontal Petri plates in the presence of 24-epibrassinolide (EBL, 100 nM), one of the most active natural brassinosteroids, lay flat on the agar surface, thus demonstrating compromised negative gravitropism. On the contrary, brassinazole (BRZ, 1 μM), an inhibitor of brassinosteroid biosynthesis, increased the negative gravitropism of hypocotyls. EBL had no effect on the gravitropic bending of hypocotyls in gravistimulated plants grown on vertical Petri plates, while BRZ increased it. EBL and BRZ treatments had opposite effects on cell wall mechanics estimated by the creep method. EBL increased the wall creep at pH 6, which is consistent with the weakening of cell walls and their disability to support the weight of hypocotyls against gravity. BRZ decreased creep under these conditions, thus increasing the wall strength. EBL did not influence the wall monosaccharide composition, cellulose and uronic acid content but led to disordered cellulose microfibril orientation in the outer epidermal wall as revealed with the Pontamine S4B dye. This could explain the effect of EBL on gravitropism, because wild type plants treated with oryzalin (250 nM) and *pom2-4* mutants, both having disordered microfibrils, also demonstrated a decrease in the percentage of standing hypocotyls. BRZ stimulated gravitropism independently of cellulose orientation, as it increased the percentage of standing hypocotyls in oryzalin-treated wild type and *pom2-4* plants to 100%. The BRZ effect on gravitropism was accompanied by a decrease in cellulose and mannose content, and an increase in non-cellulosic glucose, which is consistent with changes in cell wall mannans and/or cellulose crystallinity. The involvement of mannans in the control of gravitropism was confirmed using a triple *csla2csla3csla9* mutant deficient in this class of polysaccharides that demonstrated increased gravitropic bending. BRZ also changed the pattern of epidermal cell expansion along the axis of hypocotyls greatly diminishing the growth rate of three most basal epidermal cells. This effect may result

from decreased cell wall loosening at the base of hypocotyls, which will also contribute to the stimulation of negative gravitropism in the presence of BRZ.
In conclusion, we found that EBL and BRZ exert opposite effects on gravitropism influencing different aspects of cell wall biology. Thus, they are not antagonists in their mechanism of action on gravitropism at the cell wall level. Several cell wall-related mechanisms may affect the negative gravitropism of Arabidopsis hypocotyls: changes in the mannan content, cellulose orientation and crystallinity, and the different pattern of loosening at the base of hypocotyls.

Long remote carry out of light and gene activity signals in plant seedling

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Light provides the energy source for photosynthesis and it also act as an information source to recognize a plant about its environment. The aim of this investigation is study transferring to long distance light signal transduction and carrying out genes expression in relationship of between stem and root apeces of seedlings. The study reveals that a light signal is transferring from top stem till root system. In case X-rays irradiated of the top of seedling in not irradiated root system were observed changes expression of genes.
The changes in genes *DHN3, PCNA, SAMS2, TubA1, CDC2* expression activities in shielded from the X-ray plant roots Pisum sativum L. under the influence of an alleged distant signal generated by the irradiated shoot part of plants. Changes in gene *TubA1* expression in pea seedlings by radiation was negligible and there was no significant difference compared with control with a reduction processes in postradiation term period, indicating that a stable operation of the gene under various experimental conditions. The increase in gene *SAMS2* activity in the remote distance due to its ability to encode S-adenosyl L-methionine synthase and denote methyl groups to DNA methyltransferase. Under influence radiation was increase of the gene expression of the stem in compare of the screened root of the gene *PCNA*, which is closely linked to the nuclear antigen of cellular proliferation. That is, in remote distance unexposed parts a gene is active of the part seedling, compared to irradiated bodies appear brighter due to intense compensatory processes. The product of the gene *DHN3* synthesis is dehydrin which shows the protective effect of plants on the stress factor, the evidence suggests that it contributes to a more rapid return to normal state of the plants where irradiated stem and root screened, than completely irradiated plants. On the 4th day after irradiation the top part of the pea plant activity of genes *PCNA* and *CDC2* in the roots of the plants in comparative to the underground part completely irradiated plants increased 180 times and the activity of a gene *SAMS2* - 23 times, the gene *TubA1* expression is increased only by 44%, and the activity of gene *DHN3* is reduced by almost 18 times. Therefore an expression level of the gene *TubA 1*, using as a control shown to be so stabile.
In the unexposed parts of the plant expression of the genes are in a complicated dependence on the dose received by the exposed part of the plant, and it is possible that the signaling molecules can be siRNA, since the signal effect is nonspecific and can easily be transferred from the plant apoplastic and/or symplastic passing to the long distance. Thus, we obtained an effective test assess for the study of long remote processes occurring in the plant when exposed by visible light or ionizing radiation.

SESSION 10

ROS, RNS and Neurotransmitter Signalling

The influence of oxidative stress on size of halo during powdery mildew fungus penetration

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Plants are often exposed to stress conditions that adversely affect their growth, development or productivity. Reactive oxygen species, as ubiquitous messengers of stress responses, play a signaling role in adaptive processes. In the infected plant cell, the accumulation of reactive oxygen species can cause rapid death of plant cells, blocking the development of the pathogen. It is known that hydrogen peroxide accumulates at the sites of contact of the host plant and pathogen between epidermal cells undergoing an hypersensitive response and the subjacent mesophyll cells.
The characteristic feature of the pathogenesis of *Erysiphe graminis* DC. f. sp. *tritici* March on the leaves of wheat is the formation of halo. The halo can be observed on the surface of epidermis of wheat in form of area of chemical changes the cell wall. Halo occurs at the contact points of primary and secondary germ tubes of powdery mildews fungus with epidermal cells of wheat leaves as a paired structure (small and large halo). An interesting fact is the detection of hydrogen peroxide in the halo area.
To model oxidative stress wheat leaves were treated with 10 mM of hydrogen peroxide. Detached leaves (*Triticum aestivum* L.) were infected with *E. graminis tritici* and incubated in Petri dishes on reagent solutions for 2-6 days. Halo in control looking like concentric blue or rose colored circles 60–120 mkm in diameter. Treatment with hydrogen peroxide increased the average size of paired and single halo. Moreover, among single largest halo was found up to 3 fractions anomalously large halo which were not detected in control. Among largest halo was observed as a sharp change in morphology - slightly colored violet-gray halo and uncolored halo with gray, purple or blue rings.
Our previous studies have demonstrated that treatment with hydrogen peroxide inhibited development of pathogen colonies and increased the number of abnormal appressoria. Thus these observations suggest that increase of abnormal appressoria and the changes in halo morphology and size is a result of oxidative stress. Apparently, the reason for variability of halo may be local features interaction of a pathogen with some plant cells, including local differences in metabolism of active oxygen species.

Nitric oxide and melatonin crosstalk with reactive oxygen species scavenging enzymes in modulating abiotic stress tolerance

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Understanding the physiological and biochemical basis of abiotic stress tolerance in plants is one of the major aspects of research aiming to enhance plant productivity in arid and semi-arid cultivated lands world over. Salinity results in a significant reduction in seedling growth and development, and leads to enhanced generation of reactive oxygen species (ROS). In the recent past, crucial independent roles of various enzymatic antioxidant mechanisms and their regulatory molecules, like nitric oxide (NO) and melatonin, in modulating plant growth under salt stress, have been investigated. There is still, however, no clear understanding of the biochemical signaling pathway(s) associated with salt stress tolerance acquisition in plant cells. Present work aims to examine the possible regulatory roles of melatonin as an antioxidant through its crosstalk with NO, as an early and long-distance salt-sensing mechanism in salt stress tolerance in sunflower (*Helianthus annuus*) seedlings as a model system. Nitric oxide (NO) acts in a concentration or redox-dependent manner to counter oxidative stress either by directly acting as an antioxidant through scavenging reactive oxygen species (ROS), such as superoxide anions ($O_2^{-\cdot}$), to form peroxynitrite ($ONOO^-$) or by acting as a signaling molecule, thereby altering gene expression. NO can

interact with different metal centres in proteins, such as zinc-sulphur clusters, heme-iron, iron-sulphur clusters, and copper, resulting in the formation of stable metal-nitrosyl complexes or production of biochemical signals, which lead to modifications of protein structure/function. Thiols (ferrous iron-thiol complex and nitrosothiols) are also involved in the mobilization of NO by their binding to NO and its transport to the sites of actions where nitrosothiols release NO into the target cells. S-nitrosoglutathione (GSNO) also transnitrosylates proteins and is also an $NO^{\cdot}$ reservoir and a long distance signaling molecule. Tyrosine nitration of proteins is a biomarker of nitrosative stress as it can lead to activation or inhibition of target proteins. Immunolocalization of heme oxygenase (HO-1) distribution in sunflower seedling cotyledons has provided new information on its differential spatial distribution as a long distance signaling response to NaCl stress. NaCl-modulated HO-1 activity also correlates with endogenous NO accumulation in seedling cotyledons. Thus, NO positively modulates HO-1 activity in sunflower seedling cotyledons. NO positively modulates HO-1 activity probably by its interaction with the heme group of HO-1. Interesting data on the regulation of abiotic stress tolerance in plants by melatonin has accumulated in the recent past. This indoleamine possesses antioxidative and growth-inducing properties, thus proving beneficial for stress acclimatization. NO acts as a modulator of melatonin accumulation in seedling cotyledons as a long-distance signaling response. Melatonin inhibits NO, $O_2^{\cdot-}$ and $ONOO^{-}$ production, extent of tyrsoine nitration and also modulates the activities of SOD isoforms, thereby implicating it in modulating the deleterious effects of ROS and RNS. Melatonin and nitric oxide (NO) also differentially ameliorate salt stress effects by modulating GR activity, GSH content and GSH/GSSG ratio in seedling cotyledons. A correlation is evident in the NaCl–sensitized modulation of GSH content and GR activity by melatonin. GSH content is down regulated by NO provided as sodium nitroprusside (SNP). SNP lowers the activity of hydroxyindole-O-methyltransferase (HIOMT) - a key regulatory enzyme in melatonin biosynthesis in control seedlings whereas its activity is upregulated in salt-stressed cotyledons. A crosstalk between NO and melatonin thus modulates GR activity and GSH content to regulate ROS content during seedling growth under salt stress. Present observations provide further directions to investigate the possible roles of N-nitrosomelatonin and S-nitrosoglutathione as long distance signaling molecules in plants.

Hydrogen peroxide as a link between the redox state of the plastoquinone pool and systemic acquired acclimation to environmental light conditions

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Environmental light conditions lead to alterations in organization of the pigment-protein assemblies in chloroplasts. These alterations result from systemic acquired acclimations, namely, short-term (state transitions) and long-term (modulation of the PSII antenna size due to downregulation of the antenna proteins biosynthesis). Both are strictly regulated by the redox state of the plastoquinone pool (PQ pool) of the thylakoid membranes.

The methodological approach, which allows estimating the state transitions occurrence in whole leaf has been settled. We have shown that the duration of actinic light is an important factor, which gives the main impact on the accuracy of the state transitions estimation. The reliable estimation of the complete transition of state1 to state2 requires at least ten minutes of illumination prior NPQ measurements, while 20 minutes of illumination prior the chlorophyll fluorescence measurements at low temperature. In arabidopsis plants state transitions are inhibited at light intensity higher than 300 μmol quanta m^{-2} s^{-1}, whereas in barley plants state transitions proceed till much higher intensity, 800 μmol quanta m^{-2} s^{-1}.

Using arabidopsis wild type and *stn7* knockout mutant plants, the proceeding of state transitions in plants exposed to long-term illumination has been studied. The experimental evidence revealed that state transitions ceased to proceed during the first day after transferring of plants to high light (HL, 450 μmol quanta m^{-2} s^{-1}). However, the reappearance of state transitions was observed on the third day of the treatment of plants by HL. The correlation between the level of hydrogen peroxide in leaves, the PQ-pool redox state and the state transitions proceeding was further investigated.

Using barley plants, we have gained insight into the molecular signal reflecting the PQ-pool redox state that regulates the antenna size of PSII in long term. It is known that HL conditions experienced for more than three days lead to a reduction of the PSII light-harvesting antenna size. We have shown that the reduction of the PSII antenna size was hampered in HL (1000 μmol quanta m^{-2} s^{-1}) in the leaves possessing the high reduction level of the PQ pool but having the low hydrogen peroxide content. These conditions were achieved by the incubation of barley leaves under HL illumination in a medium contained catalase. It was also observed that the peroxidase activity of leaves was significantly higher in HL in the presence of catalase with respect to the control leaves, showing that catalase-contained

medium not only results in the lack of the signal but also the medium possess the protective action for the leaves.
Incubation of barley leaves in LL (100 μmol quanta m^{-2} s^{-1}) in a medium contained hydrogen peroxide allowed reaching the hydrogen peroxide level in leaves similar to that observed in HL, while the reduction level of the PQ pool remained unchanged. These conditions lead to the reduced PSII antenna size, which was comparable to that observed under high light conditions.
It has been concluded that hydrogen peroxide, which is produced with the involvement of the PQ pool, influences the redox activity of STN7 kinase that switches the signalling pathways, which initiate the short-term and the long-term acclimation of plants to various light conditions.
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Using a self-referencing biosensor and kinematics to assay physiological differences mediated by altered apyrase expression in transgenic roots

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In animal cells extracellular nucleotides are a hormone-like signal regulating many important physiological responses. Recent evidence suggests that extracellular ATP and ADP (eATP and eADP) play a similar role in regulating plant growth and development. For example, in root hairs there is a biphasic response to applied ATP and ADP, with lower levels promoting growth and higher levels inhibiting growth. Apyrases are enzymes that can hydrolyze ATP and ADP, and some are ectoapyrases that function to regulate the concentration of eATP and eADP. In Arabidopsis the apyrase gene family is made up of seven members. Two of these Arabidopsis apyrases, Apy1 and Apy2, appear to function as ectoapyrases by regulating the level of eATP and eADP. In this study we used an enzyme-based microsensor to measure the concentrations of eATP outside of roots in wild-type and apyrase transgenic seedlings. We found differences in eATP levels in different developmental zones in wild-type roots as well as differences between wild-type and apyrase transgenic roots. We also examined elemental growth for the wild-type and transgenic primary roots and documented differences in their growth patterns. We will present details of these analyses and discuss the significance of these findings.

Signaling in *Allium cepa* cells for elicitation of oxidative burst and defense responses

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Plants have a net of signal systems for defense against biotic stress. After recognition of a pathogen plants induce a series of defense responses including generation of active oxygen species (AOS), accumulation of PR proteins and phytoalexin (PA) synthesis [1]. However, the function and mechanism of generation of AOS and their role in later plant defense responses induction is still unclear. The aim of this study was to examine signaling for elicitation of oxidative burst and PA synthesis in elicitor-treated onion cells.
Suspension-cultured onion cells (*Allium cepa* L.) cv. Skvirsky obtained from callus tissue. The proteinaceous biotic elicitor was prepared from fungal pathogen *B. cinerea* and added to 2 ml of cell suspension, which was then incubated for 48 h to elicit PA synthesis. The amount of both onion PA (tsibulins 1D and 2D) was determined by HPLC. Generation of superoxide in elicitor-treated onion cells was measured by chemiluminescence of lucigenin, which is specific for $O_2^{\cdot-}$ [2].
The data obtained suggest that onion cells responded to biotic elicitor in the presence of DDC, superoxide-dismutase (SOD) inhibitor (1mM), by dramatically increasing of $O_2^{\cdot-}$ production. Perhaps the amount of SOD or other antioxidant system in onion cells was enough to dismutate all $O_2^{\cdot-}$ to H_2O_2 nevertheless superoxide concentration increased after elicitor recognition. The question aroused which enzymes were responsible for the inducible production of $O_2^{\cdot-}$

Since molecular machinery underlying the oxidative burst might be conserved between mammalian and plant cells, we examined the involvement of NADPH oxidase that is known to generate of $O_2^{\cdot-}$ for oxidative burst in neutrophils. DPI, a suicide substrate inhibitor, which directly inhibits mammalian NADPH oxidase activity by binding to the flavoprotein component of the oxidase complex was used to study the involvement of NADPH oxidase in the formation of superoxide. It turned out that at concentration of 50 μM inhibited 83% the chemiluminescence elicited by the elicitor.

To elucidate Ca^{2+} involvement in the induction of AOS in *A. cepa* we analyzed the effect of various reagents, which are known to change the level of cytosolic Ca^{2+} in other systems. The formation of $O_2^{\cdot-}$ by elicitor-treated onion cells was diminished when the culture medium was depleted of calcium. When cells were washed in medium containing 5 mM EGTA, a specific chelating agent for Ca^{2+}, a significant decrease in formation of $O_2^{\cdot-}$ was observed. Verapamil is known to block potential-dependent Ca^{2+} channels. Addition of verapamil 20 min before elicitor treatment had significant inhibitory effect on $O_2^{\cdot-}$ generation (51 % inhibition).

As a calmodulin is a ubiquitous calcium-sensor protein that is an important transducer of Ca^{2+} signal we examined its role in signal transduction for activation of AOS-producing systems. Both calmodulin antagonists (TFP and W-7) completely inhibited elicitor-induced $O_2^{\cdot-}$ generation in onion cells.

Taken together, the inhibiting action of Ca^{2+} chelator, inhibitor of voltage-dependent Ca^{2+} channels and calmodulin antagonists suggest the involvement of Ca^{2+} and calmodulin in the activation of $O_2^{\cdot-}$-generating systems. Probably, Ca^{2+} can modulate the NADPH oxidase via direct or indirect route.

Elicitation of PA synthesis therefore represents a useful model for studing of signal perception and transduction mechanisms. In elicitor-treated onion cells the increase of $O_2^{\cdot-}$ concentration is required for the elicitation of plant defense mechanisms. Our previous studies had shown that the increase of cAMP level in onion cells stimulated PA synthesis and callose deposition [3]. Present results suggest that formation of $O_2^{\cdot-}$ as observed in elicited cells appears to depend on both Ca^{2+} and calmodulin. Although a possible source of superoxide in *A. cepa* cells is likely to be an oxidase with a flavoprotein subunit (inhibited by DPI), a relative contributions of various oxidases remains to be clarified.

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Exogenous nitric oxide and arginine mitigates drought stress in wheat seedlings through the regulation of water balance and modulation of antioxidant defense and glyoxalase systems

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To investigate the possible regulatory role of NO in relation to the regulation of antioxidant defense and glyoxalase systems, the wheat (*Tricicum aestivum* L. cv. Pradip) seedlings were grown in semi-hydroponic medium. A set of 6-d-old seedlings was pretreated with 500 μM sodium nitroprusside (SNP – a NO donor) and L-agrinine (Arg) for 24 h. Two levels of drought stress (15 and 30% PEG) were imposed separately as well as in SNP- or Arg-pretreated seedlings and grown further for 48 h. Drought resulted in oxidative stress as evidenced by increased lipid peroxidation (MDA, malondialdehyde), H_2O_2 content and $O_2^{\bullet-}$ generation rate, higher lipoxygenase (LOX) activity and MG level. Drought stress decreased leaf chlorophyll (chl) and relative water content (RWC); increased proline (Pro); decreased ascorbate (AsA); increased endogenous GSH and glutathione disulfide (GSSG) content; increased the GSH/GSSG ratio; increased ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), and superoxide dismutase (SOD) activities; decreased activities of dehydroascorbate reductase (DHAR) and catalase (CAT) while glutathione *S*-transferase (GST) activity was not changed. Activities of glyoxalase I (Gly I) was not changed but glyoxalase II (Gly II) increased due to drought. Compared to drought alone, exogenous SNP and Arg supplementation with drought reduced LOX activity, improved chl, AsA, GSH, GSH/GSSG ratio and activities of antioxidant defense and glyoxalase enzymes helped to reduce oxidative stress and MG toxicity. Comparatively, Arg showed

better results than SNP. These results were also associated with the enhanced level of endogenous NO and associated enzymes. The results indicate that the exogenous application of NO donor increased the tolerance of the wheat plants to drought-induced oxidative damages.

Formation of reduced reactive oxygen species in a photosynthetic electron transport chain, and their signaling role

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A consensual view is that photosynthetic electron transport chains (PETC) are the sensitive detectors of changes in the environment. According to thermodynamics reasons, the oxygen reduction in the PETC (Mehler reaction) of higher plants, green algae, and cyanobacteria is inevitable process in the nowaday Earth atmosphere. *In vivo,* oxygen reduction occurs simultaneously with $NADP^+$ reduction, and in various species, the oxygen can accept up to 60% of electrons going through PETC. It was established, that the primary product of O_2 molecule reduction in PETC is superoxide anion radical. The reduced reactive oxygen species, superoxide radical, $O_2^{\bullet-}$, and hydrogen peroxide, H_2O_2, formed from it are recognized molecules participating in redox signaling. A thorough insight into the details of forming these molecules can promote understanding the ways of signals transmitting from PETC to other plant systems.

Owing to their redox-potentials the various PETC components can reduce O_2 molecules as outside thylakoid membrane, so inside it. Using the molecules with different lipophilicity, which, reacting with superoxide radicals, give ESR-detectible signal, we in the experiments with thylakoids got direct evidence that PETC components can generate $O_2^{\bullet-}$ in hydrophilic area, *i.e.* in the stroma *in vivo,* and within the hydrophobic membrane phase [1].

The analysis of numerous early studies as well as our direct measurements proved that the contribution of ferredoxin to O_2 reduction is negligible during $NADP^+$ reduction, while O_2 reduction by membrane-bound PETC components is even so still significant, increasing with increase of light intensity [2]. It was discovered that oxygen reduction can occur in the plastoquinone pool (PQ-pool); the peculiarities of pH-dependence of this process showed that reduction of O_2 molecules to $O_2^{\bullet-}$ is executed there by plastosemiquinone in the reaction proceeding at the membrane/water interphase [3, 4].

In PSI, O_2 reduction occurs simultaneously by phylloquinones occupying A_1-sites and by F_A/F_B, the terminal electron transfer cofactors; in the first process $O_2^{\bullet-}$ is generated within thylakoid membrane, and in the second one it is generated in the medium [5]. In moderate and high light, phyllosemiquinone has been stated to be the principal PETC component that produces $O_2^{\bullet-}$ [6].

Not all superoxide radicals produced in PETC can be detected in the thylakoids; and the share of the non-detectable superoxides increases with increasing actinic light intensity [7]. Moreover, an increase of light intensity results in an increase of amount of H_2O_2 molecules produced within thylakoid membrane rather than in hydrophilic area [8]. Collation of these data with previous finding that the PQ-pool contribution to the total oxygen reduction in PETC increased with increasing actinic light intensity, exceeding appreciably its contribution to $O_2^{\bullet-}$ production [9], and the peculiarities of PQ-pool oxidation kinetics following illumination [10], both led to conclusion that the production of H_2O_2 within the membrane occurred in the reaction of superoxide radical with PQH_2.

Thus, redox state of PQ-pool can control the production of H_2O_2 molecules. It is hypothesized that the hydrogen peroxide molecules being formed with PQH_2 participation are the main transmitters by which the redox state of the PQ-pool provides its regulating effect on the PSII antenna size under various light conditions.

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Role of low-molecular-weight thiols during development of effective and ineffective legume-*Rhizobium* symbiosis

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The ascorbate-glutathione pathway is one of the main antioxidant mechanisms in plants and, in particular, in nitrogen-fixing nodules of legumes, where numerous processes facilitate the production of reactive oxygen species. To date a lot of evidence demonstrating its importance for establishment of symbiosis and nitrogen fixation have been accumulated. Glutathione is a major low-molecular-weight thiol in plants, involving in cell cycle regulation, development, sulfur transport and storage, stress responses, as well as in the other processes. Reduced glutathione (GSH) is continuously oxidized to a disulphide form (GSSG) that is recycled to GSH by NADPH-dependent glutathione reductase (GR). The GSH : GSSG ratio affects the cellular redox state, which regulates numerous processes in cell. Some of the effects of GSH are connected with its role in signalling transduction, whereas the others are linked to hormone and secondary metabolite synthesis. In legumes, the structural homolog, homoglutathione (hGSH), may partially or completely replace GSH. In legume nodules there is a high positive correlation between nitrogenase activity, GSH and hGSH content.
A series of pea ineffective mutants blocked at different stages of nodule development and the corresponding wild-type SGE were used to reveal the spatial localization of GSH, its biosynthetic pathway's enzymes (γ-glutamylcysteine synthetase, glutathione synthetase) and GR in symbiotic nodule. Immunolocalization revealed GSH in the active meristematic cells, vascular bundles and uninfected cells of parenchyma in all analyzed genotypes. In wild-type nodules, GSH was also observed in cells of the infection and nitrogen-fixing zones. GSH was also localized in infected cells of the mutant *sym40* (an ortholog of *Medicago truncatula EFD*), which is characterized by hypertrophied infection droplets. The mutant *sym33* (an ortholog of *M. truncatula IPD3* and *Lotus japonicus CYCLOPS*) is characterized by 'locked' suberinized infection threads and the absence of bacterial release into the host-cell cytoplasm. In such infection threads label was associated with bacteria inside them. However, occasionally bacterial release can take place in some cells. In this case, GSH accumulation was observed in cells containing bacteroides. In all analyzed variants, the most intensive labeling was observed around juvenile bacteroides, indicating the important role of GSH during the stage of bacterial release from infection droplets into the plant cell cytoplasm. In addition, GSH may be involved in the defense reactions, which are highly expressed in mutants *sym33* and *sym40*.
Biosynthetic pathway's enzymes and GR have a similar spatial localization with GSH and in wild-type nodules the most intensive labeling was observed in the meristematic and nitrogen fixing zones. In addition, an intensive accumulation of GR in nodule tissues was also observed. It can be assumed, that for effective nitrogen fixation it is more essential to maintain the specific cellular redox state, rather than the synthesis of GSH *de novo*.
We also examined transcriptional activity of *GSH1* (γ-glutamylcysteine synthetase), *GSHS* (glutathione synthetase) and *hGSHS* (homoglutathione synthetase) in nodules. In all mutants the considerable increase in *GSH1* gene expression as compared with wild-type was observed. The highest expression levels of the genes *GSHS* and *hGSHS* were observed in mutants *sym40* and *sym33*, correspondingly. These observations suggest the presence of distinct regulatory mechanisms for the *GSHS* and *hGSHS* genes, but, most importantly, they provide support for the different role of GSH and hGSH in nodules.
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Nitric oxide-Peroxidase crosstalk in the signaling events associated with salt stress tolerance in sunflower seedlings

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Sunflower is a moderately salt-tolerant crop. In order to achieve salt tolerance, plants are equipped with an elaborate network for scavenging elevated reactive oxygen species (ROS) generation during salt stress. It is composed of antioxidant enzymes and antioxidants, responsible for maintaining levels of ROS. Catalase (CAT) is suggested to be involved in mass scavenging of H_2O_2 whereas peroxidase (POD) is involved in fine regulation of H_2O_2 accumulation in cells. PODs occur in three forms in plants, namely

soluble, covalently bound and ionically bound. Soluble PODs are found in the cell cytoplasm while the ionically and covalently bound forms are associated with cell wall and some cell organelles. Peroxidases (POD) are heme-containing enzymes. It is well known that NO acts as an iron ligand in heme proteins, thereby leading to their activation or inhibition. A few reports have documented the ability of NO to bind to the heme moiety of peroxidases and reversibly inhibit enzyme activity. Present investigations provide interesting and extensive results about a NO-POD crosstalk accompanying the modulation of salt stress tolerance in sunflower seedlings. Elevating NO availability in the roots of sunflower seedlings through an external source (SNP) leads to an increase in the specific activity of POD in a SNP concentration-dependent manner. Comparing the POD protein levels in SNP and cPTIO treated seedlings, it is evident that salt stress leads to enhanced accumulation of the protein. But irrespective of that, POD activity is positively regulated by NO. These combined observations from the impact of SNP, cPTIO and aminoguanidine (NOS inhibitor) indicate that: 1. (Endogenous) nitric oxide positively modulates POD activity in seedling roots; 2. POD activity modulation by NO is not sensitized by the presence or absence of salt stress; 3. Salt stress, however, results in the accumulation of POD proteins (not activity). Cotyledons derived from seedlings raised in variable concentrations (125-500 μM) of SNP, on the other hand, exhibited a better concentration-dependent upregulation of POD activity. A clear difference is seen in the sensitivity of the cotyledon tissue to the elevated concentrations of nitric oxide in control seedling cotyledons as well as those derived from salt-stressed conditions. Zymographic observations of root homogenates indicate a better sensitivity of the root system to cPTIO in terms of its ability to nullify the effect of NO on POD activity than in case of seedling cotyledons. Thus, it is evident that putative NOS activity inhibition is differentially modulated by salt stress in seedling cotyledons and its inhibition correlates with lowering of POD activity. Nitric oxide can lead to various post-translational modifications of proteins (tyrosine nitration, S-nitrosylation and metal nitrosylation) thereby, modulating their activity. Further investigations are required to know the exact mechanism of regulation of POD activity by NO. Proteomic analysis of SNP treated sunflower seedling cotyledons would further reveal POD content in seedling cotyledons upon SNP treatment as compared to control.

Congruence between PM H^+-ATPase and NADPH oxidase during root growth: A necessary probability

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Having central roles in regulation of growth & development and defence against biotic/abiotic challenges, plasma membrane (PM) located H^+-ATPase and NADPH oxidase (NOX) have emerged as keystone enzymes with extensive signalling properties (both up- and downstream). Cell elongation growth by wall relaxation is one of the several convergence points, where H^+-ATPase incurs apoplastic acidification (optimum for expansin) and NOX provides $O_2^{\bullet -}$ to produce $OH^{\bullet}$ radical (by Haber-Weiss reaction) necessary for wall-polysaccharide cleavage. Experiments, aimed to find the possible synchronization, revealed a putative feed-forward loop in 2-day grown roots of *Vigna radiata* (L.) Wilczek. Disruption of cross-PM proton gradient by CCCP and specific inhibition of PM H^+-ATPase by sodium ortho-vanadate revealed that proton gradient, generated due to its regulated extrusion by PM H^+-ATPase, is crucial for NOX-dependent ROS metabolism. Apart from histolocalization studies (decreased NBT staining), spectrophotometric (XTT) assays of both bathing medium of intact tissues and root tissue extracts showed lower superoxide production than control. *In-gel* Native PAGE assay for NOX corroborated the $O_2^{\bullet -}$ lowering with reduced functioning of NOX. Conversely, both spectrophotometric and Native PAGE assays demonstrated the diminution of PM H^+-ATPase activity in response to different ROS scavengers (KI and $CuCl_2$) and NOX inhibitor ($ZnCl_2$), with the effect of $CuCl_2$ being the most pronounced. Repressing effects of Ca^{+2} homeostasis antagonists (especially La^{+3}, Ca^{+2} channel blocker and EGTA, Ca^{+2} chelator) indicate a prerequisite of threshold $[Ca^{+2}]_{cyt}$, maintained by Ca^{+2} influx through PM, for both the enzymes. Ethylene, regulator of an unusual Ca^{+2} channel (HACC) distinct from the common ones, seemed to function upstream of the enzymes after studying the effects of Ethrel, $AgNO_3$ and $CoCl_2$. Since unlike animal NOX, the plant versions do not possess proton channel activity, deftly harmonized functioning of PM H^+-ATPase and NOX at certain phases e.g. root growth, appears to be well justified.

Possible role of JIN1/MYC2 transcript-factor in induction of salt resistance in *Arabidopsis* plants by nitric oxide

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JIN1/MYC2 transcript-factor is one of the proteins participating in stress signaling in plants. It is considered that JIN1/MYC2 provides the transduction not only jasmonate-induced signals to genome, but also unites the transduction of ABA- and jasmonate-dependent stress signals, and participates in the formation of different plant adaptive responses against drought and salinity (Ton et al., 2009; Lackman et al., 2011). Based on the data obtained with bioinformatics methods, the conclusion about the possible participation of nitric oxide (NO) in the regulation of gene expression of MYC family was made (Palmieri et al., 2008). However, the experimental data about the role of JIN1/MYC2 transcript-factor in the realization of NO-dependent physiological effects in plant cells are still absent.

We have documented the investigation of NO donor (sodium nitroprusside, SNP) effects on the elicitation of salt resistance in *Arabidopsis thaliana* L. wild-type (Col-0) and *jin1* mutants (defective in jasmonate signaling).

Four-week *A. thaliana* plants were grown in the water culture using Gibeaut medium (Gibeaut et al., 1997) at 24/18°C (day/night), 6000 lx illumination and 10 h photoperiod. 0.5 mM SNP and/or 0.5 mM PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, NO scavenger) were added to the nutrient medium and afterwards plants incubated during 24 h. Then plants were transferred to the nutrient medium without specified additives and part of them was exposed to the salt stress by the addition of 200 mM NaCl. After plants incubation during 24 h with sodium chloride the medium was replaced with ordinary Gibeaut medium.

It turned out that *jin1* mutants were more sensitive to the salt stress as compared to wild-type plants. SNP treatment has stabilized the biomass accumulation in wild-type plants under salt stress, but did not have any positive effect on biomass of *jin1* mutants.

The content of lipid peroxidation product malonic dialdehyde (MDA) was increased in the leaves of both plant genotypes under salt stress. At the same time SNP treatment significantly softened the effect of oxidative stress manifestation in wild-type plants, but practically did not influence the MDA content after salt stress in *jin1* mutants leaves.

After the salt stress the content of photosynthetic pigments in both *Col-0* and *jin1* plants was decreased. SNP pretreatment promoted the saving of chlorophyll content in the leaves of *Col-0* plants and did not has a positive impact on their content in *jin1* mutants under the conditions of salt stress. Positive effect of SNP treatment on wild type plants was leveled if the plants were pretreated with PTIO.

SNP treatment caused rather small, but marked increase of superoxide dismutase (SOD) activity in *Col-0* plants and did not have an impact on this activity in *jin1* genotype. The salt stress induced the decrease of SOD activity in plants of both genotypes, however it was more remarkable in *jin1* mutants. SNP treatment of wild-type plants promoted the preservation of enzyme activity closed to the control level, but did not affect its value in *jin1* mutants. Catalase activity in plants of both genotypes under the salt stress was decreased. SNP treatment prevented this effect in wild-type but not in *jin1* plants. The activity of guaiacol peroxidase (GPX) in wild-type plants under the salt stress exceeded its value in *jin1* plants. Moreover SNP treatment of *Col-0* plants stimulated additional increase of GPX activity in leaves under the salt stress.

The data obtained suggest that NO donor caused the increase of salt resistance in wild-type *A. thaliana* plants via induction of their antioxidant enzyme system. Such positive effects of SNP were not revealed in *jin1* mutants. That allowed us to make an assumption about direct/indirect participation of JIN1/MYC2 transcript-factor in NO-dependent signaling for plant defense responses against the salt stress.

The role of glutathione and superoxide dismutase in the adaptive response of barley root cells to cadmium

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It has been suggested that one of the important reactions, determining the resistance of plants to cadmium, is the ability of cells to respond quickly to a sharp reduction of glutathione (GSH), in consequence of the use of its molecules on the synthesis of phytochelatins (PCs), by activation of genes involved in its synthesis, as well as by the synthesis *de novo* of antioxidant enzymes. Based on this, the aim of our research was to investigate the adaptive response of root cells to a sharp decrease in the level of GSH, caused by cadmium, in barley seedlings differing in the age and the resistance to the metal.
Experiments were performed with barley (Hordeum vulgare L., cv. Zazerskii 85). Seeds were grown on a modified nutrient solution. When seedlings reached the age of either three or seven days, in experimental variants nutrient solution was supplemented with 100 μM cadmium. After 4 days of exposure, GSH and PC content in the roots of seedlings was determined by HPLC, the overall activity of superoxide dismutase (SOD) was assayed spectrophotometrically, and the level of *HvGS* and *HvCu/ZnSOD* genes transcripts (encoding superoxide dismutase and GSH synthetase, respectively) was determined by RT-PCR analysis.
It was found, that in the presence of cadmium, in roots of 3-day-old (less resistant to cadmium, as established earlier) and 7-day-old (more stable) seedlings significantly increased the amount of PCs. Simultaneously drastically decreased GSH content, leading to a change in redox potential of cells, and oxidative stress. However, further in 7-day-old seedlings the number of GSH was readily increased and became rather higher than in control plants. Also activation of *HvGS* was observed, indicating increased synthesis of GSH. In addition, the number of *HvCu/ZnSOD* gene transcripts was increased, indicating the launch of antioxidant defense mechanisms, and moreover the activity of SOD was raised. In contrast, in 3-day-old seedlings, after exposure with cadmium, the level of transcripts of these genes was not changed. At the same time GSH content was reduced, though the SOD activity was somewhat increased, presumably due to activation of existing molecules.
On the base of these data, we can conclude that higher tolerance of 7-day-old barley seedlings to cadmium is associated, at least in part, with the ability to root cells quickly respond to the reduction of the content of GSH, due of high metal concentration, by gene expression activation (in particular, *HvGS*), responsible for GSH synthesis, as well as genes that control the synthesis of antioxidant enzymes, including SOD, thus allowing recovery of the redox balance of the cells and protect them from oxidative stress. In 3-day-old seedlings, in the presence of cadmium, amount of GSH does not increase, which is obviously associated with the retardation (or complete blocking) of its synthesis. Also the transcription level of antioxidant enzyme genes does not change, which leads to accumulation of active oxygen species in the cells and, apparently, may be one of the reason of their lower resistance to the metal.

Ni(Fe)-ARD Acireductone Dioxygenases participate in the biosynthesis of ethylene. Tyr-fragment role as a regulatory factor in the formation of neurotransmitters. AFM-study self-organization of model Ni-Tyr(or PhOH) complexes and morphology of mitochondria

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The methionine salvage pathway (MSP) plays a critical role in regulating a number of important metabolites in prokaryotes and eukaryotes. Two Ni(Fe)-ARD Acireductone Dioxygenases are involved in methionine salvage path, which regulates the cell cycle aspects. Structural and functional differences between the two enzymes ARDs depend on the metal ion bound at the active site of the enzyme. Methionine (and ethylene also) forms only as a result of the oxygenation of Acireductone with dioxygen, catalyzed by Fe-ARD. But methionine does not form as a result of Ni-ARD action. However, an important function of Ni-ARD was established: as a result of Ni-enzyme action carbon monoxide forms. CO, like nitrogen oxide, NO, is representative of a new class of neurotransmitters.
We assumed that one of the reasons for the different activity of Ni(Fe)-ARD in the functioning of enzymes in relation to the common substrates (Acireductone and dioxygen) can be the association of

catalyst in various macrostructure due to intermolecular H-bonds and the other no covalent interactions. The possibility of the formation of stable supramolecular nanostructures based on structural and functional models of Ni(Fe)-ARD: $Ni_2(OAc)_3(acac)MP{\bullet}2H_2O$ (MP = N-methylpirrolidon-2), $Fe^{III}_x(acac)_y18C6_m(H_2O)_n$ (18C6=18-crown-6) we first confirmed by AFM. We previously suggested that, in the case of Ni-ARD, Tyr-moiety, if it participates in the mechanism, can reduce the activity of Ni-ARD. As we established the inclusion of PhOH in model complex $Ni(acac)_2{\cdot}MP$ results in its stabilizing. In this case, the acac– ligand (analogue of Acireductone) does not undergo O_2-dependent transformation. Also the stability of triple complexes $Ni(acac)_2{\bullet}L^2{\bullet}PhOH$ seems to be due to the formation of stable to oxidation of supramolecular macrostructures due to intra- and intermolecular H-bonds. Formation of supramolecular macrostructures due to intermolecular H-bonds on the basis of complexes of $\{Ni(acac)_2{\cdot}MP{\cdot}PhOH\}$ that we set by the AFM method, testifies in favor of the hypothesis of the role of phenol-containing fragment. Recently with AFM we got evidence for involvement of Tyr-fragment as a regulatory factor in the mechanism of Ni-ARD action. Self-assembly based on triple systems that included L-Tyrosine (Tyr) as extra ligand, $\{Ni^{II}(acac)_2+MP+Tyr\}$, formed on a surface of modified silicone, we observed first.

We discovered with AFM method that intermolecular hydrogen bonds were formed also between surfaces of modified silicon and the applied on it biological objects, which were used in the capacity of samples. These samples were mitochondria 6-day-old pea seedlings, which were fixed with 2% glutaraldehyde. Through AFM technique, we found swelling of the mitochondria of these seedlings in the conditions of the 2-day shortage of water. This swelling of mitochondria was, apparently, caused by lipid peroxidation. Oxidative modification of lipid membranes of mitochondria leads to changes in energy metabolism and influences the physiological indicators, primarily the growth processes.

Thus, AFM technique allows not only to explore the nature of chemical bonds, but also to trace morphological changes in biological structures.

NO-donors and L-Arginine Stimulate L-tryptophan Decarboxylase Activity, Tryptamine and Vincamine Accumulation in Callus Cultures of *Vinca minor* L

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Vinca minor L. (Apocynaceae) is an important medicinal plant producing terpenoid indole alkaloids (TIA) which are used as cerebral vasodilators. The major alkaloid of *V. minor* leaves is the monoterpenoid indole alkaloid vincamine (Smeyers et al. 1991). L-tryptophan decarboxylase (TDC, 4.1.1.28) is the key enzyme involved in the biosynthesis of terpenoid indole alkaloids (Facchini et al. 2000). TDC catalyzes the transformation of L-tryptophan to tryptamine in the early stages of TIA biosynthesis. TIA biosynthesis in *V. minor* remains poorly investigated and regulation of TDC activity and endogenous tryptamine content in plants or in cultured *V. minor* cells is not studied. Meanwhile, much attention is given to the plant cell culture as a tool for the production and biosynthesis investigation of important TIA. However a limited number of papers have been published on *in vitro* culture of *V. minor*. This research has focused on the involvement of nitric oxide signaling system in the regulation of *V. minor* TIA biosynthesis.

The callus culture was obtained from leaves explants. Calli were grown on Murashige and Skoog basal medium containing 6-furfurylaminopurine (kinetin) and a-naphthaleneacetic acid (NAA) in growth chamber. Biochemical analyses were carried out on 5-years callus cultures. TDC activity and tryptamine accumulation was assayed as described by Sangwan et al. (1998). Vincamine was detected by HPLC-MS/MS.

TDC activity and tryptamine accumulation were observed in callus cultures of *Vinca minor* L. The optimal conditions for the enzyme activity, tryptamine and vincamine accumulation were identified. TDC activity was evaluated during the culture cycle as well as the accumulation of tryptamine and TIA. The TDC activity was shown to depend on conditions of callus cultivation and cultures age. TDC activity and tryptamine accumulation in callus cultures were strongly stimulated by light.

NO donors sodium nitroprusside (SNP) and S-nitrosoglutation (GSNO) were shown to regulate TDC activity and tryptamine accumulation in callus cultures of *V. minor*. The increase in NO donor concentration (1-50 μM) had stimulatory effect on TDC activity, while higher concentrations of SNP resulted in the inhibition of enzyme activity. Tryptamine accumulation was shown to be directly proportional to the level of TDC activity. Moreover, stimulating effects of NO donors on dry biomass,

total protein and vincamine accumulation were demonstrated. L-arginine (0.1-1 mM) caused a similar stimulation of TDC activity, endogenous tryptamine content and vincamine accumulation. The increase in L-arginine concentration had also stimulatory effect on callus culture growth processes. Effects of SNP, GSNO and L-arginine were inhibited by the NO scavenger cPTIO.
By this means, our data suggest that the enzymatic production of NO (NOS-like activity) might be involved in the regulation of TDC activity and TIA biosynthesis in callus cultures of *V. minor*.

The occurrence and the biosynthesis of a 'neurotransmitter' acetylcholine in *Urtica thunbergiana* (Nettle)

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A wide range of plant species have been shown to accumulate a neurotransmitter acetylcholine (ACh) in the last couple of decades. Numerous reports also demonstrate that exogenous application of ACh affects gravitropism, tolerance to salinity and various other aspects of plant physiology. Since plants do not have authentic neuronal networks found in mammals, these results support the view that ACh might play novel biological roles beyond neuronal signals in plants. However, no genes that are responsible for the biosynthesis of ACh have been identified from plants so far. In addition, ACh has been detected generally by electro-physiology assays, radio-immuno assays or liquid chromatography electro-chemical detection (LC-ECD), which all detects ACh only indirectly. These collectively impose questions as to whether plants indeed biosynthesize and accumulate ACh. To this end, we developed analytical methods of ACh using liquid chromatography tandem mass spectrometry (LC-MS/MS) and electrospray ionization-orbitrap Fourier transform mass spectrometry (ESI-orbitrap FT-MS). These high resolution analytical methods allowed us to quantitate ACh in plant tissues, therefore validating that ACh does exist in plants. We then biochemically purified choline-*O*-acetyltransferase (ChAT) to near homogeneity from crude extracts of *Urtica thunbergiana* (Nettle) which is known to accumulate considerable amount of ACh. Through peptide MS fingerprinting and degenerate PCR approaches, we successfully identified a putative cDNA sequence of *Urtica* ChAT (UtChAT). Phylogenetic analysis revealed that the putative UtChAT is a member of acyltransferase superfamily and constitutes a novel clade with unknown biological function. While genes homologous to the putative UtChAT are well conserved among plants, no animal genes exhibit appreciable similarity to UtChAT. Altogether, our results provide strong evidence that *Urtica* is able to biosynthesize ACh by a plant-specific ChAT, and that ACh in animal and plant kingdoms might have occurred independently through convergent evolution.

Detection of acetylcholine in higher plants by LC-MS/MS analysis

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A wide range of bacterial and plant species have been documented to accumulate a neurotransmitter acetylcholine (ACh) in the last couple of decades. These reports suggest that ACh might exhibit novel biological activities beyond neuronal signals. However, i) genes that are responsible for the biosynthesis of ACh have not been reported so far, and ii) ACh has been detected mostly by radio-immuno assays or liquid chromatography electro-chemical detection (LC-ECD), both of which have some drawbacks in their specificity. These collectively impose questions as to whether plants indeed biosynthesize and accumulate ACh. Here we developed various analytical methods including liquid chromatography tandem mass spectrometry (LC-MS/MS) and electrospray ionization-orbitrap Fourier transform mass spectrometry (ESI-orbitrap FT-MS), and applied them to quantitate ACh within tissues of *Arabidopsis thaliana*. The data showed that the level of ACh in Arabidopsis was highest in seed, followed by root and cotyledon. Moreover, when Arabidopsis seedlings were grown on agar medium containing ACh showed root hair inhibition. Altogether, our data clearly indicate that ACh is accumulated within plant tissue, and that the biological role that ACh plays might be related to root hair development.

Physiological and biochemical mechanism of spermine-induced cadmium toxicity tolerance in mung bean (*Vigna radiata* L.) seedlings

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Cadmium (Cd) is persistent hazardous heavy metal severely damaging plants. The role of exogenous spermine (Spm, a polyamine, PA) in alleviating Cd toxicity effects was studied. This study investigates that exogenously applied Spm (0.25 mM) reduces Cd content, accumulation and translocation in root and shoot of mung bean (*Vigna radiata* L. cv. BARI Mung-2) plants exposed to Cd (1 mM and 1.5 mM $CdCl_2$). Spm supplementation increased phytochelatin content which reduced Cd content. Spermine application reduced the oxidative damage induced by Cd as evidenced from the reduction of H_2O_2 content, $O_2^{\bullet-}$ generation rate, LOX activity and lipid peroxidation, and also reflected from the reduction of spots of H_2O_2 and $O_2^{\bullet-}$ from leaves investigated by histochemical detection, compared to control. Cadmium provoked oxidative damage was relieved by reactive oxygen species (ROS) scavenging nature of Spm and other endogenous PAs. Exogenous Spm induced increase in non-enzymatic andtioxidants (ascorbate, AsA and glutathione, GSH) and activities of antioxidant enzymes (such as: superoxide dismutase, SOD; catalase, CAT, glutathione *S*-transferase, GST; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR and glutathione reductase, GR) which altogether were involved in scavenging ROS. The cytotoxicity of methylglyoxal (MG) was also reduced by exogenous Spm due to the enhancement of glyoxalase system enzymes and components. Through osmoregulation Spm maintained a better water status of Cd affected mung bean seedlings and prevented chlorophyll damage. Exogenous Spm also modulated endogenous free PAs level which might have roles in improving physiological parameters. The overall Spm induced Cd toxicity tolerance was reflected through improved growth.

Nitric oxide has concentration-dependent effects on the cell cycle acting via EIN2 in *Arabidopsis thaliana* cultivated cells

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Cell division is one of the most conserved processes operating in both animal and plant cells; its integrity being essential for an organism's form and function. Many studies in mammalian cells have shown the importance of nitric oxide (NO) to cell cycle progression. However, whilst there is much literature describing the biological role of NO in plants, its potential role in regulating the cell cycle has remained obscure. There have been, nonetheless, some few notable studies on the role of NO as a regulator of cell cycle in plants. Taken together, these studies suggest that NO does affect cell cycle progression in a concentration-dependent manner; exerting a stimulatory effect at low concentrations but having the opposite effect at higher ones. However, the check-point(s) regulated by NO and molecular mechanism are unknown. Establishing the mechanism(s) could be facilitated through the use of cultivated *Arabidopsis thaliana* cells. These avoid problems associated with intact plants where cell cycle progression could be modified by the presence of diverse intercellular interactions and complex developmental programs. Moreover, these systems are further complicated by the fact that the

meristematic region is small in size and its cells are heterogeneous. Considering the above circumstances, we studied NO effects on the cell cycle of cultivated *A. thaliana* wild type (Col-0) and ethylene-insensitive mutant *ein2-1* cells. Cultivated cells of *ein2-1* mutant were included in this study since interrelations between NO and ethylene biosynthesis depends on physiological context. Additionally, it has been shown that ethylene has an inconsistent role(s) in cell cycle progression. In contrast to the root apical meristem, the cultivated cells are free of organismic control and functional heterogeneity which is an undoubted advantage in working with them.

We compared suspension cells derived from *A. thaliana* Col-0 and *ein2-1* plants to determine the contribution of ethylene and NO to proliferation control. Both NO and ethylene were produced mainly during the first five days, but ethylene generation was reduced and NO increased in *ein2-1.* Nitric oxide effects were assessed by the addition of the NO-donor sodium nitroprusside (SNP) to cultivated cells. At all SNP concentrations, ethylene synthesis was significantly diminished in Col-0 and unchanged in *ein2-1*. Flow cytometry analysis showed that in both cultures, low SNP concentrations (5 and 20 μM for Col-0 and 5 μM for *ein2-1*) increased S-phase cell number favored G1/S transition but at higher SNP concentrations (100 and 500 μM) cell cycle progression was arrested at G1/S and G2/M in Col-0 and *ein2-1*, respectively, reflecting EIN2 requirement for these effects. Concomitantly, there was some increase in *CYCA2;3* expression with 5 and 20 μM SNP but at higher concentrations *CYCD3;1*, *CDKA;1* and *CDKB2;1* expression were suppressed. An inhibitory effect of higher SNP concentrations on *CYCB1;1* and *CDKB2;1* expression was revealed in *ein2-1* but the expression of *CYCA2;*3 and *CYCD3;1* was not significantly altered. Based on these observations it is possible to derive a preliminary model for NO/ethylene effects on the cell cycle. The number of S-phase cells is maintained in an EIN2-dependent manner most likely via an EIN2-linked initiation of NO production. At low concentrations of exogenous NO an increase of the S-phase cells and both *CYCA2;3* and *CYCB1;1* expression is apparent. Under higher exogenous NO, there are reductions in the expression of *CYCA2;3, CYCD3;1, CYCB1;1, CDKA;1,* and *CDKB2;1* with corresponding arrest in certain cell cycle phases. Clearly, such a simple model requires further characterization but it does generate several hypotheses on which further studies can be based. Thus, we suggested that NO acts as a concentration-dependent modulator of cell cycle progression with a dependency on EIN2 for both NO production and appropriate regulatory function.

Zinc alleviates cadmium toxicity in *Chara australis*: Role for reactive oxygen species?

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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. 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. Our previous attempts to show an increase in ROS in response to heavy metal exposure was unsuccessful. We show that early changes in ROS are detectable, but are transient and small. While they may have a role in early signaling events leading to responses in to they do not appear to be playing a role in zinc-mediated proection. We propose that glutathione has an effect through other pathways, possibly by chelation of Cd directly or via increased synthesis of phytochelatins.

Blue-light dependent reactive oxygen species formation in *Eutrema salsugineum* callus cells may be alternative signaling mechanism

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Light is the most important factor in the environment necessary for plant growth and development. The change of the light spectral composition affects not only on photosynthesis, but also in other related processes, such as the formation of secondary metabolites and proteins, and the formation of the antioxidant status of cells. In recent years, there is evidence of the involvement of blue light receptors cryptochromes in the generation of reactive oxygen species. We used the plant callus lines extremophile *Eutrema salsugineum*. This model system allows studying signaling and regulatory properties of light separately from its photosynthetic component, and regardless of the effect of the whole plant physiology. The influence of narrow-band blue light on activity of PSII, the H_2O_2 content, malondialdehyde and activity of key antioxidant enzymes in *E. salsugineum* callus cells was studied. The 50-watt LED-matrix emitting with peak in 450 nm was used as blue-light source. The callus cells growth under white 50-watt LED-matrix was used as a reference control. All of experimental was carried out in light-proof boxes at 16-h photoperiod.
The calli grown in control and test conditions were practically identical in morphology and growth rate. However, staining dyes on cell viability showed a high proportion of dead cells in the experimental cells grown under blue light.
Photosynthetic performances were carrying out on the basis of chlorophyll fluorescence induction curves. The number of active PSII reaction centers in callus cells grown in blue light was less than that of the control group of calluses. The values of quantum yields: maximum (Fv / Fm), with QA electron transfer to QB until the final acceptors PSI was significantly lower in the experimental calluses, than in the control. When grown on a blue light efficiency of energy dissipation into heat PSII (antenna) was higher compared to cells grown in control conditions.
In the calli grown under blue light the H_2O_2 content was 2 times higher than that under white light and caused an increase in the malondialdehyde content in 3.5 times. In the cells of the experimental variant was observed increased ascorbate peroxidase and peroxidase activity but superoxide dismutase activity was decreased. This indicates that the callus culture under blue light influenced on aerobic metabolic processes by altering the balance of reactive oxygen species, which in turn affect the activity of antioxidant enzymes.
The findings indicate that blue light had a strong prooxidant effect on *E. salsugineum* callus cells, which could be a consequence of intracellular signaling, likely initiators which can be cryptochromes.
The reported study was partially funded by RFBR according to the research project No 16-34-01269 mol_a.

Insect herbivory-elicited GABA accumulation in plants – a player in defense?

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The non-proteinogenic amino acid γ-aminobutyric acid (GABA) is present in all organisms analyzed so far. In invertebrates GABA acts as neurotransmitter; in plants different functions are under discussion. Among others, its involvement in abiotic stress reactions and as defensive compound against feeding insects is suggested. GABA is synthesized from glutamate by glutamate decarboxylases (GAD) and degraded by GABA-transaminases. Here, in *Arabidopsis thaliana*, *gad1/2* double mutants showing reduced GABA contents as well as GABA-enriched triple mutants (*gad1/2 x pop2-5*) were generated and

employed for a systematic study of GABA induction, accumulation and related effects in *Arabidopsis* leaves upon herbivory. Results demonstrate that GABA accumulation is stimulated by insect feeding-like wounding by a robotic caterpillar, MecWorm, as well as by real insect (*Spodoptera littoralis*) herbivory. Both higher GABA level in plant tissue and in artificial insect diet reduced the growth and performance of feeding larvae indicating a role in plant defense. GABA enrichment in the attacked plant occurs not only in the challenged but also in adjacent leaf. Furthermore this induced response is neither depending on herbivore defense-related phytohormones, jasmonates, nor is jasmonate induction depending on the presence of GABA. Thus, in plants the rapid accumulation of GABA very likely represents a general, direct and systemic defense reaction against insect herbivores.

Regulation of pathways of intercellular water transfer in *Zea mays* roots in response to induction of oxidative stress

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The objective of current work is the elicitation of common reaction of elements of water transport system of plants in response to various exogenous inductors of oxidative stress.
The method was developed and implemented for selective registration of symplastic water transfer (via plasmodesmata) in intact plants directly during the application of inductors of oxidative stress.
The closure of aquaporins in the zone of treatment in response to the induction of oxidative stress is a common reaction for inductors with various mechanisms of action. Water conductance of the symplastic pathway decreases under the induction of oxidative stress in roots of intact plants. In the zone of root distant from the zone of treatment with inductors of oxidative stress the activation of total intercellular water transport is observed. The novel capabilities for the examination of the reaction of water transport system in roots of intact plants in response to the induction of oxidative stress are demonstrated.

Nitric oxide-Heme oxygenase crosstalk accompanying salt stress in sunflower seedlings

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Investigations on the characterization of iron signals in plants are a recent area of research. Heme is one of the major iron sensors in plants. Free heme can be highly toxic since it catalyzes the formation of reactive oxygen species (ROS). Catabolism of free heme is carried out by heme oxygenase (HOs; EC 1.14.99.3) which uses heme both as a prosthetic group and as a substrate. Salt stress adaptation in sunflower seedlings accompanies accumulation of ROS and enhanced production of nitric oxide (NO). Immunolocalization of heme oxygenase (HO-1) distribution in sunflower seedling cotyledons by confocal laser scanning microscopic (CLSM) imaging has provided new information on its differential spatial distribution as a long distance signaling response to NaCl stress. HO-1 activity is abundant in the specialized cells around the secretory canals (SCs) in seedling cotyledons. Abundance of tyrosine nitrated proteins has also been observed in the same cells in salt stressed seedling cotyledons. Present findings highlight a link among distribution of HO-1 expression, abundance of tyrosine nitrated proteins and mitochondria in specialized cells around the SCs as a mechanism of salt stress tolerance in sunflower seedlings. Enhanced spatial distribution of HO-1 in response to NaCl stress in seedling cotyledons also coincides with an increase in specific activity of HO-1 in NaCl stressed conditions. HO-1 activity is further enhanced by hemin (HO-1 inducer) both in the absence or presence of NaCl stress and is inhibited by zinc protoporphyrin. NaCl-modulated HO-1 activity also correlates with endogenous NO accumulation in the cotyledons. Increased NO accumulation by hemin treatment coincides with enhanced activity of HO-1 both in control and NaCl stress conditions. Thus, NO positively modulates HO-1 activity in sunflower seedling cotyledons and NaCl stress antagonizes NO action in this respect. NO probably positively modulates HO-1 activity by its interaction/binding with its heme group. Enhanced NO accumulation in seedling cotyledons both in the absence or presence of iron in the growth

medium, in response to NaCl stress, indicates a probable link between endogenous NO, NaCl stress and iron-homeostasis by way of modulation of HO-1 activity in sunflower seedlings.

Calcium and nitric oxide-dependent nuclear accumulation of cytosolic glyceraldehyde-3-phosphate dehydrogenase in response to long chain bases in tobacco BY-2 cells

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Dihydrosphingosine (DHS), one of the most abundant free sphingoid Long Chain Base (LCB) in plants, is known to induce a calcium-dependent programmed cell death in plants. In tobacco BY-2 cells, DHS triggers a rapid production of H_2O_2 and nitric oxide (NO). Recently, by analogy to what is known in the animal field, plant cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPC), a ubiquitous enzyme involved in glycolysis, has been suggested to fulfill other functions associated with its oxidative post-translational modifications such as *S*-nitrosylation on cysteine residues. In particular, in mammals, stress signals inducing NO production promote *S*-nitrosylation of GAPC and its subsequent translocation into the nucleus where the protein participates in the establishment of apoptosis. In the present study, we investigated the behavior of GAPC in tobacco BY-2 cells treated with DHS. We found that upon DHS treatment, an *S*-nitrosylated form of GAPC accumulated in the nucleus. This accumulation was dependent on NO production. Two genes encoding GAPCs, namely *Nt(BY-2)GAPC1* and *Nt(BY-2)GAPC2*, were cloned. Transient expression of Nt(BY-2)GAPCs-GFP chimeric constructs indicated that both proteins localized in the cytopsol as well as in the nucleus. Mutating into serine the two cysteine residues, supposed to be *S*-nitrosylated in response to DHS, did not modify the localization of the proteins, suggesting that *S*-nitrosylation of GAPCs is not necessary to their nuclear relocalization. Interestingly, using Förster resonance energy transfer experiments, we showed that Nt(BY-2)GAPCs interact with nucleic acids in the nucleus. When GAPCs were mutated on their cysteine residues, their interaction with nucleic acid was abolished, suggesting a role of GAPCs in the protection of nucleic acid against oxidative stress.

Pea (*Pisum sativum* L.) nodule development: reactive oxygen species, antioxidants and phytohormones

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Pea (*Pisum sativum* L.) symbiotic nodule is a useful tool to study nodule formation at the biochemical, genetic, and physiological levels.
We have studied the involvement of reactive oxygen species, antioxidants and phytohormones during nodule development. A set of pea mutants was used with mutations in genes *sym33* ('locked' suberized infection threads), *sym40* (hypertrophied infection droplets, premature nodule senescence), *sym26*, *sym27* (premature nodule senescence) and *sym42* (wall of infection threads are filled with callose, and premature nodule senescence).
Cytochemical analysis has revealed that hydrogen peroxide is involved in maturation of the infection thread wall and release of bacteria into the host cell cytoplasm from infection droplets. Hydrogen peroxide was also observed around degrading bacteroids during nodule senescence. In nodules of ineffective mutants in the genes *sym40* and *sym42* the amount of hydrogen peroxide was increased in comparison with wild-type.
(Homo)glutathione ((h)GSH) is a major low-molecular-weight thiol in plants, involving in antioxidant defense in symbiotic nodules. Immunolocalization demonstrated that the most intensive labeling was observed around juvenile bacteroids, indicating the important role of GSH during the stage of bacterial release from infection droplets into the plant cell cytoplasm. Analysis of transcriptional activity was performed for genes involved in (h)GSH synthesis in nodules: *GSH1* (γ-glutamylcysteine synthetase), *GSHS* (glutathione synthetase) and *hGSHS* (homoglutathione synthetase). In mutants in the genes

sym40 and *sym33* a considerable increase in *GSH1* expression was observed, as compared with wild-type. The highest expression levels of the genes *GSHS* and *hGSHS* were observed in mutants *sym40* and *sym33*, correspondingly. These observations suggest the presence of distinct regulatory mechanisms for the *GSHS* and *hGSHS* genes, but, most importantly, they provide support for the different role of GSH and hGSH in nodules.

Immunolocalization of gibberellic acid showed the maximum of the label in the infection zone and the nitrogen-fixation zone and a decline in the senescent zone. In mutants in the genes *sym26* and *sym27*, demonstrating premature nodule senescence, the intensity of gibberellic acid labeling was significantly lower than in wild-type nodules. The mutants were also characterized with increased expression of the gene encoding gibberellin 2-β-hydroxylase (*PsGAOx2*), an enzyme involved in gibberellic acid catabolism. These data indicate possible involvement of gibberellic acid in the negative regulation of pea nodule senescence.

Immunolocalization of cytokinins showed their preferential localization in wild-type nodules in the meristem and the infection zone. For a mutant in the gene *sym33,* label was associated with cells in the base of the nodule. It seems that cytokinin is involved in cell differentiation in nodule, probably triggering cell endoreduplication.

Thus, it was shown that different mechanisms, including reactive oxygen species, antioxidants and phytohormones are involved in pea symbiotic development.

The study was supported by Russian Science Foundation [14-24-00135], Russian Fund for Basic Research [14-04-00383].

Hydrogen peroxide distribution in pea (*Pisum sativum* L.) symbiotic nodule

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Comparative cytochemical analysis was used to investigate differences in hydrogen peroxide distribution, as revealed by conversion of cerium chloride into precipates of cerium perhydroxide in thin sections of nodule tissue. The study involved symbiotic nodules of pea initial line SGE and cv. Finale and mutants SGEFix$^-$-1 (*sym40*), SGEFix$^-$-2 (*sym33*), and RisFixV (*sym42*), correspondingly. In the initial line SGE, precipitates of cerium perhydroxide were deposited in the walls of infection threads and in adjacent material in the luminal matrix.

In mutant SGEFix$^-$-1 (*sym40*), an increased deposition of cerium perhydroxide was observed in the matrix of hypertrophied infection droplets, around bacteria contained in infection threads and also around juvenile bacteroids. The observed pattern of hydrogen peroxide distribution indicates that bacteria in infected cells of mutant nodules are exposed to a stronger oxidative stress compared with nodules of the initial line.

In mutant SGEFix$^-$-2 (*sym33*), peroxide was less abundant in the infection thread wall and lumen but was associated with the enclosed rhizobial cells. In addition, precipitates of cerium perhydroxide were observed in the form of large droplets at the outer surface of the infection thread wall.

Compared to cv. Finale, infection threads of RisFixV (*sym42*) showed increased levels of cell wall peroxide, detectable by cerium chloride precipitation. Peroxide was also associated with released bacteroids in this mutant.

Previously, it was shown that mutations in pea symbiotic genes *sym33* and *sym40* lead to suberin depositions in ineffective nodules, and in mutant *sym42* to callose depositions in infection thread and host cell walls (1). The increased amount of cerium perhydroxide precipitates in nodules of the mutants *sym40* and *sym42*, as well as the changed pattern in mutant *sym33* can be the reason of the increased defense reactions in nodules of these mutants.

The study was supported by Russian Science Foundation [16-16-10035].

1. Ivanova et al. 2015. Protoplasma 252:1505-1517.

Workshops and Satellite Sessions

Exploring the power of parallel measurements of electron transport, CO_2 and H_2O in plant leaves

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Over the years, increasing population, growing industrialization, expanding agriculture and rising standards of living have pushed up the demand for water while the prospects for increasing the supply are limited. We need to learn to use water more efficiently. There continues to be a significant if not increasing amount of research effort focused on the improvement of water use efficiency in plants of economic importance. At the cutting edge of that research are studies which characterize and evaluate the potential for manipulating CO_2 diffusion within the leaf (mesophyll conductance; g_m).
There are several methods for determining g_m. All of the methods involve measurements of gas exchange of CO_2 and H_2O. With the introduction of commercial instruments which measure CO_2 and H_2O flux in parallel with estimates of electron transport using fluorometry research in this area has become more common. However there are a number of assumptions and pitfalls to these measurements. The author will discuss some of these assumptions and how the new LI-COR photosynthesis system (LI-6800) was designed to minimize violations of the assumptions.

Automatic sample preparation for TEM using Leica Microsystems instruments

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The preparation of plant material for study using transmission electron microscopy (TEM) is a very time- and labour-consuming task. The most steps involve hazardous reagents and have to be performed manually according to time schedule. Automatic processing of samples provides more streamlined and efficient laboratory workflow, time and cost savings, minimizes contact with hazardous reagents, improves user safety in the laboratory and provides reproducible results. Leica Microsystems offers solutions for both room and low temperature sample preparation TEM.
Leica tissue processor EM AMW is highly automated system for chemical fixation, processing, embedding and polymerization. The combination of microwave chamber and automatic reagent changer minimizes user effort and significantly reduces processing time to about 5 hours. Some artifacts of chemical and even cryo fixation can be avoided due short fixation and embedding time [1,2]. The preservation of antigenicity is similar to that from conventionally prepared samples [2].
For sample processing at low temperatures the freeze substitution and low temperature embedding system Leica EM AFS2 was designed. Its combination with the Leica EM FSP (freeze substitution processor), an automatic reagent handling system, prevents unfavourable temperature rises in specimen chamber.
Tissue processors and other Leica automatic instruments for TEM sample preparation may help to overcome the limitations of traditional TEM preparation and make this complicated but informative method applicable for screening procedures.
The Leica Microsystems instruments for TEM as well as histological sample preparation, light and confocal microscopes will be demonstrated at the Workshop "Cell biology techniques in plant signaling studies" in the research resource center «Molecular and cell technologies» of St. Petersburg State University».
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Nikon confocal systems: what's new in advanced fluorescent microscopy

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Nikon is a world-renowned brand, firmly established as a market leader in optical instrumentation. With almost 100 years expertise in the field, Nikon manufactures whole range of light microscopes including innovative stereomicroscopes with world's largest zoom range and high-end super resolution systems for live cell imaging.

Nikon now has a confocal microscopy solution to suit virtually every research need. Advanced fully-automated laser confocal scanning system A1+ with galvano or A1R+ with hybrid scanner enables fast imaging of highest resolution. Supersensitive GaAsP detectors allow acquiring up to 5 channels simultaneously where 4 fluorescent channels can be superimposed with high quality DIC image to aid in image analysis such as locating fluorescence labels.

Following options are especially in use for low-phototoxic high-quality plant tissue imaging:

- An objective inverter: unique brand feature, not only can convert inverted to upright microscope configuration but allows excellent confocal visualization in any special orientation
- Fast 32-channel spectral detector: allows accurate real-time spectral unmixing with maximum performance in discrimination of closely overlapping fluorescence spectra and the elimination of autofluorescence
- V-filtering function: filterless intensity adjustment is possible by selecting desired spectral ranges from 32 channels that match the spectrum of the fluorescence probe in use and combining them to perform the filtering function
- Image resolution enhancement: additional software package NIS-Elements C-ER allows up to 1.5-fold improvement in axial and lateral resolution as well as pushing up signal-to-noise ratio by advanced denoising function

In my talk I'd like to show how the listed features combined in individual approach can help to improve quality of resulted data.

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