SESSION 5:
Signaling netwoks

THE PARTICIPATION OF NITRATE ION IN SHOOT TO ROOT SIGNALING

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It is usually observed that enhanced nitrate nutrition of plants leads to suppression of assimilate export from the source leaves and decreased root to shoot weight ratio, and this decrease in shoot/root ratio is instantly dependent on nitrate content in a shoot. Now there is an active search for signal mechanisms integrating root and shoot responses to changes in the plant nitrogen supply.

Our investigations have shown that enhanced nitrate fertilization results in enhanced sucrose hydrolysis in the apoplast. Apoplast in many plants is an intermediate compartment through which sucrose moves to the phloem. Hexoses formed in the process of sucrose hydrolysis are not able to be loaded into the phloem and that is why assimilate export decreases. But these investigations were carried out in a whole plant and it was not possible to understand if enhanced sucrose hydrolysis in the leaves was connected with nitrate incoming into the leaf apoplast or nitrate metabolism in the plant roots. That is why we cut off plant roots and fed nitrate solutions directly into the shoot apoplast through the transpiration water stream. One hour after the beginning of solutions feeding a photosynthetic chamber was put on the middle shoot part through which $^{14}CO_2$ was blown during 3 min.

It was found that nitrate feeding into the apoplast led to the same effects as nitrate feeding through the soil (decreased $^{14}CO_2$ assimilation, decreased labeled sucrose to hexose ratio and changes in photosynthetic carbon metabolism). Feeding water or reduced nitrogen had not such impact on photosynthesis. The action of nitrate directly depended on its concentration, and cation at the nitrate did not play a crucial role. The investigation of ^{14}C distribution throughout the plant in 30 min and 3 h after exposure of the plant middle part to $^{14}CO_2$ showed that nitrate introduction into the apoplast led to inhibition of assimilate export. Concurrently ^{14}C-sucrose gradually accumulated in the leaves, whereas in leaves of control plants (water feeding) the relative content of ^{14}C-sucrose after the initial increase (30 min) decreased with time. Autoradiography of the whole leaves showed that when water was incorporated to the apoplast ^{14}C-assimilates concentrated in large veins, from which they were then exported, while nitrate incorporation led to ^{14}C-assimilate accumulation outside the large veins. Analysis of minor phloem vein ultrastructure one hour after the beginning of nitrate feeding showed that in response to nitrate appearing in the apoplast a large central vacuole is formed in companion cells while in usual conditions and upon water feeding there is no large vacuoles in these cells. Thus, a place of the labeled sucrose accumulation could be large vacuoles formed in companion cells.

Because in numerous investigations of Y.V. Gamalei and his colleagues a formation of such a vacuole in companion cells was observed when assimilate export was inhibited by putting a cold collar on the petiole an assumption can be made that nitrate feeding into the apoplast also initially creates some hindrances to assimilate transport through the phloem or assimilate transport from companion cells to sieve elements. In this case enhancement of apoplastic invertase activity in the presence of nitrates could be a consequence of sucrose accumulation in the apoplast. Hexoses formed in the process of sucrose hydrolysis are not able to be loaded into the phloem and have to come back into the mesophyll cells and this will stimulate the growth of leaves. Concurrently, decreased sucrose incoming to the roots will result in reduces root growth.

ROLE OF AUXIN-MEDIATED FORMATION OF REACTIVE OXYGEN SPECIES IN GRAVITY-DIRECTED PLANT GROWTH

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Reactive oxygen species (ROS) are one of the major signal intermediates that take part in regulation of many processes in plant cell metabolism. At system level they take part in growth and development of plant tissues that is mediated by plant hormones, particularly auxin. Interdependence between auxin and ROS level was investigated. Recent evidences support hypotheses that ROS and auxin also play one of the major roles in the mechanism of gravity-oriented growth (gravitropism). According to this relevant information we suggest that specific bilateral localization of ROS in formative tissue either a part of signal perception mechanism or signal transduction cascade, determines endurance and direction of plant growth reaction. Nitroblue tetrazole (NBT) was used to visualize oxygen radicals in order to determine in our model system (*Zea maize* coleoptiles) effect of ROS on gravity bending. We have discovered that decrease of ROS level reduces ability to adequately percept changes of gravity vector by plant tissues. Strong reducing of ROS level resulted in significantly less sensibility to gravity, but did not affect curvature angle of stem. Responsivity can be partly reversed with IAA treatment. Using the agar blocks for local exogenous application of IAA significantly increased curvature angle of stem or initiated this process in the absence of gravity change stimulus. When using low concentrations of hydrogen peroxide for local application, which is a substrate for generating of oxygen radicals by peroxidases, also had influences on gravitropic bending. These results may indicate that perception of gravity stimulus depends on ROS level in competent tissues. IAA can stimulate producing of oxygen radicals, however, high concentrations of IAA inhibit gravitropic response. It may be evidence that ROS play different functions in plant tissues that depend on their localization. It is also possible that initiation of growth curvature required lateral stem gradient of ROS.

This work was supported by grant of National Space Agency of Ukraine – N4/IBNASU-2005

HYDROGEN PEROXIDE-PROMOTED STOMATAL CLOSURE IN ARABIDOPSIS GUARD CELLS IS MEDIATED BY cGMP

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H_2O_2 is known to be involved in the signaling pathway by which abscisic acid (ABA) brings about stomatal closure. Application of H_2O_2 inhibits stomatal opening and promotes stomatal closure. It was observed previously that ABA-induced stomatal closure is likely to involve cyclic guanosine 3',5'-monophosphate (cGMP) along with H_2O_2, NO, Ca^{2+}, cADPR, protein kinases and protein phosphatases. However it was not established whether cGMP is downstream of H_2O_2 action.

We showed that H_2O_2 induced the closure of preopened stomata in wild type Columbia-2 and Landsberg *erecta* plants. The guanylyl cyclase inhibitor LY 83583 entirely suppressed the H_2O_2-induced effect in wild type plants and treatment with 8-bromo-cGMP, a cell-permeable analog of cGMP, reversed the LY inhibitory influence. These data suggest that cGMP is required for H_2O_2-induced stomatal closure. Furthermore, H_2O_2 was found to induce a rapid significant increase in cGMP concentration being detectable within 30 sec, reaching a maximum within 1 min and decreasing to the prestimulation level in 5 min. Thus, the effect of H_2O_2 correlates well with cGMP dynamics. To investigate whether the H_2O_2-activated cGMP signaling pathway is calcium dependent we investigated the interaction between cGMP and Ca^{2+}. Using transgenic Arabidopsis seedlings expressing apoaequorin in cytosol, H_2O_2 was shown to induce two peaks of calcium-dependent chemiluminescence in a dose-dependent manner. The first transient peak was registered after a lag-phase of 40 sec peaked in 1 min and the second one had a lag-phase of 5-10 min reaching maximum in 19 min without reaching the basal level. Pre-treatment of seedlings with LY suppressed entirely H_2O_2-induced $[Ca^{2+}]_{cyt}$-transient while 8-bromo-cGMP reversed the LY inhibitory effect suggesting that cGMP acts upstream of the calcium transient. Moreover the [cGMP]-response to H_2O_2 appears to be faster than $[Ca^{2+}]_{cyt}$-one.

We next investigated whether the OST1 protein kinase and ABI-1 type 2C protein phosphatase are involved in H_2O_2 and cGMP dependent ABA – signaling. We found that the *ost1-4* and *abi1-1* mutations had no effect on the ability of preopened stomata to close in response to H_2O_2. *OST1* and *ost1-4* mutations did not inhibit any H_2O_2-induced increase in cGMP level indicating OST1 and ost 1-4 do not mediate H_2O_2-induced cGMP formation. Interestingly *abi1-1* mutants were impaired in [cGMP]-response to H_2O_2 indicating that abi1-1 is likely to act in H_2O_2-signaling upstream cGMP. Moreover, previous *in vitro* studies by Meinhard et al (FEBS Letters 2001, 508, 443-446; Planta 2002, 214, 775-782) have revealed that ABI1 and ABI2 activities could be inhibited by H_2O_2. However this signaling pathway is likely to be not crucial for H_2O_2-induced stomatal closure because *abi1-1* mutations had no effect on the ability of stomata to close in response to H_2O_2. In conclusion we provide the evidence that H_2O_2-promoted stomatal closure in Arabidopsis guard cells is mediated by cGMP. In addition in plant cells the H_2O_2-activated cGMP signaling pathway is calcium-dependent and involves the action of ABA-regulated protein phosphatase type 2C (abi1-1).

The work was supported by grant INTAS YSF 05-109-4746.

A REVERSE GENETICS APPROACH TARGETING THE ARABIDOPSIS MAP KINASE. KINASES REVEALS THE INVOLVEMENT OF MKK7 AND MKK9 IN MERISTEM DEVELOPMENT.

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Mitogen Activated Protein Kinase (MAP kinase) cascades, are conserved eukaryotic signalling modules that convert external signals into intracellular responses. We constructed gain of function and dominant negative versions for all the Arabidopsis MKKs, expressed in *Arabidopsis* under the control of an inducible promoter system. In the process of generating these lines we discovered that the constitutive overexpression of *MKK7* gives rise to a meristemless seedling, whereas increased *MKK9* levels lead to dwarf plants with asymmetric meristems. Due to the severity of the phenotypes we chose to concentrate on inducible expression of *MKK7* and *MKK9*. Previous data indicate the involvement of MKK7 in polar auxin transport (Dai et al, Plant Cell 2006 18(2):308-20). In our system, within two hours of induction of the *MKK7* or *MKK9* expression, increased PIN1 protein levels were detected with apolar cellular localisation. What is more, PIN1 showed ectopic expression, both in the root and shoot tissues, possibly by affecting vesicle trafficking. GUS promoter assays demonstrate that the expression domain of *MKK7* and *MKK9* expands in response to IAA and TIBA, but a lot more so in response to ACC. Moreover, whole genome CATMA microarrays were performed on seedlings expressing *MKK7* and *MKK9*. The transcription profiles generated imply that a major role of MKK7 and MKK9 action is to arrest seedling growth. Moreover, MKK7 and MKK9 like the MKK4 and MKK5, appear to be involved in ethylene biosynthesis and pathogen response.

BLUE LIGHT AND JASMONIC ACID SIGNALLING SYSTEMS INTERACTION

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The modern researchers believed that jasmonic acid (JA) is essential in the system signal transduction. At that, little is known about the role of JA in regulation of morphogenesis and balance of plant endogenous phytohormones as well as about interaction of signalling pathways induced by spectral light.

We studied the influence of blue light (BL, λ = 439 nm, 29 μmol/m^2s, 30 minutes) and jasmonic acid on morphogenesis of seedlings *A. thaliana*. Plants of *A. thaliana* ecotypes Landsberg *erecta* (L*er*) and Columbia (Col) as well as mutants *hy4* (mutated in cryptochrome), *axr1-3* (*a*u*x*in-*r*esistant) and *jar1-1* (*ja*smonate-*r*esistant) were used.

The reaction of wild type seedlings to JA in the darkness differed from mutants' one and manifested in inhibition Col, L*er* and *jar1-1*'s growth of the length of hypocotyls and also absence of the reaction of *axr1-3* seedlings defined as plants with reduced sensitivity to MeJA and other hormones. The reaction of the seedlings to JA under blue light manifested in inhibition Col, L*er*, *jar1-1*'s, and *axr1-3* growth of the length of hypocotyls.

There was no reaction of light mutant *hy4* to blue light, at that there was reaction to JA resulted in elongated growth of hypocotyls. The reaction of cotyledons to acting factors differed from hypocotyls' one.

The contents of two main phytohormones abscisic acid (ABA) and indole-acetic acid (IAA) in L*er* и *hy4* were studied. It was found that some growth responses were provoked by changes of phytohormone level and its balance. So, inhibition of the growth of hypocotyls and cotyledons of Arabidopsis of wild type L*er* under JA was caused by decrease of free IAA level and by significant increase of free ABA level not in the darkness only. For *hy4* elongation of hypocotyls in the darkness was caused by decrease of free ABA level by 3 times.

The jasmonate and auxin can use similar mechanism of signal transduction, i.e. can act by common signal messenger which influences the reaction of other phytohormones. The obtained data on simultaneous influence of JA and BL on the growth of hypocotyls allow us to believed that blue light induces the signal systems with messengers common for hormones and only *hy4* with defected sensitivity to BL did not show analogues reaction.

Basing of the results of our experiments it may be suggested that morphogenesis regulation of seedlings by JA and BL is due to changes of endogenous hormonal balance. The integration of signalling systems induced by blue light and jasmonic acid in morphogenesis of *A. thaliana* was shown.

cGMP MEDIATES ABSCISIC ACID-, NITRIC OXIDE- AND HYDROGEN PEROXIDE-INDUCED CYTOSOLIC FREE Ca^{2+} UPTAKE IN ARABIDOPSIS

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Abscisic acid, a plant growth regulator, influences plant development in variety of ways. ABA signaling is characterized by a great number of intracellular messengers. ABA enhances NO and H_2O_2 synthesis in guard cells and NO is required for ABA-induced stomatal closure. Most likely, calcium, cyclic GMP and protein kinases are downstream signaling components of H_2O_2 and NO. However, the exact interaction among the various signaling components in response to ABA, H_2O_2 and NO in plant cells remains to be established. Besides, very few measurements of actual cGMP levels in plants have been reported and potential cGMP-targets in plants have not been identified so far.

Using apoaequorin-expressing *Arabidopsis* seedlings we showed that ABA induced increase in $[Ca^{2+}]_{cyt}$ followed a lag-phase of 5 - 20 min. Gyanylyl cyclase inhibitor LY 83583 reduced ABA-mediated $[Ca^{2+}]_{cyt}$-transient strengthening the case for cGMP involvement. NO donor sodium nitroprusside (SNP) caused a single spike of calcium-dependent chemiluminescence in a dose-dependent manner. This transient increase followed a lag-phase of 2 min and lasted for 1 min. The NO scavenger carboxy-PTIO completely suppressed SNP-induced Ca^{2+}-increase. H_2O_2 resulted in two peaks of calcium-dependent chemiluminescence. The first Ca^{2+}-spike followed 40 sec lag-phase, peaked after 1 min, and the second one had 5 – 10 lag-phase min reaching maximum in 19 min. Pre-treatment of seedlings with LY 83583 decreased NO- and first H_2O_2- induced $[Ca^{2+}]_{cyt}$ increase while 8-bromo-cGMP reversed the LY inhibitory effect. Furthermore, we showed that exogenous ABA, NO and H_2O_2 caused the rapid increases in endogenous cGMP level in *Arabidopsis* seedlings. These data testify that ABA, NO and H_2O_2 bring about their effect on $[Ca^{2+}]_{cyt}$ uptake involving enhanced synthesis of cGMP which acts upstream of the calcium transient.

To elucidate the mechanism of cGMP action to bring about a Ca^{2+}-increase and further biological responses in plants we isolated cytosolic cGMP-binding proteins from *Arabidopsis* cells suggesting they to act as signaling components downstream of cGMP. cGMP-based affinity purification procedure followed by two-dimensional gel electrophoresis yielded **8** most abundant protein spots. The tryptic peptides of the isolated proteins were analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). Mascot database search resulted in unequivocal identification of isolated proteins. The nature of identified proteins was discussed in relation to their position in appropriate signaling pathways.

THE BALANCE OF PHYTOHORMONS AND RESISTANCE OF WHEAT TO FUNGI PATHOGENS

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Phytohormones play a key role in regulation of plant defense reactions to a stress factors such us infection by the pathogen. It was revealed the using of the some of phytohormones or it analogues with growth stimulate effect promote increasing of crop productivity. However the pathogens also use a phytohormons for successful colonization in plant tissue. It is necessary the mutual relation the host – pathogen take into account for research of phytohormonal balance of in infected tissue and a whole plant as it changes by the secretion of active substances by pathogens.

We was analyzed changes in phytohormons level (IAA, ABA and cytokinins) of wheat plant with different degree to stability in response to infection by bunt agent, rot root, septoriosis described a various trophy. The contents of phytohormones were defined in infected and not infected plant organs. Our researches have revealed regularities in changes of phytohormonal balance in wheat plant at pathogenesis. The infection by pathogens promotes to increasing of cytokinins level in absence of changes of IAA/ABA balance in resistant wheat plant. Moreover cytokinins content increased in plant organs space removed from place of the pathogen penetration. The cytokinins content and significant IAA/ABA fluctuations decreased by pathogens described a various trophy in susceptible wheat plant. In response to septoriosis was increased IAA level whereas ABA was increased in response to rot root in susceptible wheat plant. Our data confirm important role of cytokinins in integration of plant defense reactions as cytokinins participate in plant defense gene expression.

Thus our data indicate when use preparations with growth –stimulate and resistance –promote properties for increasing of plant productivity necessary take into account trophy of pathogens.

The work was supported by the grant of RFBR 05-04-48310 and the grant of Russian federal science and technical program 02.512.11.2051.