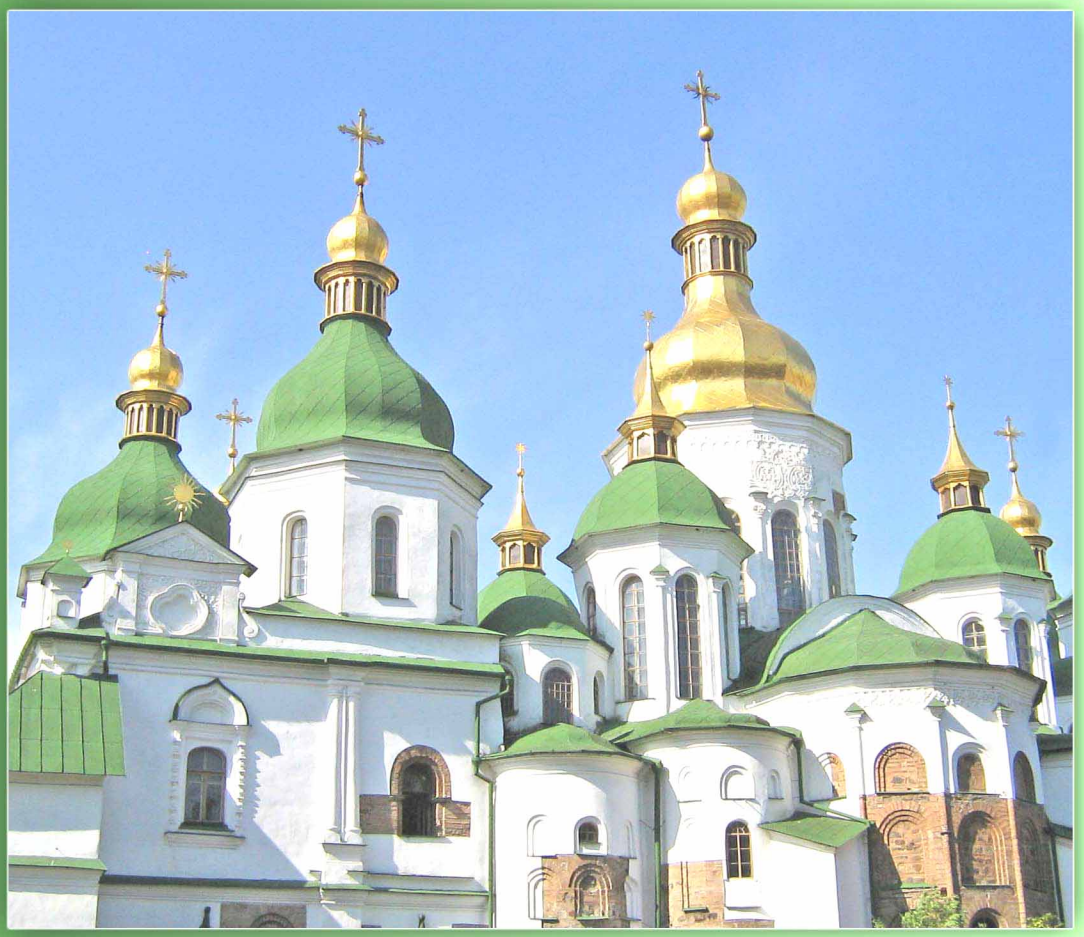
2nd International Symposium

PLANT GROWTH SUBSTANCES: INTRACELLULAR HORMONAL SIGNALING AND APPLYING IN AGRICULTURE

ABSTRACTS



8-12 October 2007 Kyiv, Ukraine

National Academy of Science of Ukraine
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Fundamental Researches State Found
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ABSTRACTS

of 2nd International Symposium

"Plant Growth Substances: Intracellular Hormonal Signaling and Applying in Agriculture"

http://www.bpci.kiev.ua/news/Conference_PGSIHS/PGSIHS_official_site.html

8-12 October, 2007
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Acknowledgements

The Organizing committee wants to express sincere thanks for support of the Symposium to the following sponsors:

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Welcome Address

PLANT GROWTH SUBSTANCES: ACHIEVEMENTS AND NEW IDEAS.

Kuchar V.P.
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On behalf of the Organizing Committee, it gives me a great pleasure to extend a warm invitation to you to participate in the 2-nd International Symposium of Plant Growth Substances to be held in Kyiv, Ukraine. The theme of the conference highlights the desire of the committee to bring together experts from a variety of fields to further knowledge and research in plant growth regulators. Our aim is to arrange a Symposium that is not only of high scientific standard, but also a socially memorable event for all participants. We are pleased to invite you to come and enjoy the science, meet your colleagues and friends and take the opportunity to establish new fruitful collaborations.

Ukrainian scientists have made a great contribution to plant hormone research. The Symposium is devoted to the 125th anniversary of N.G. Kholodny, the great phytophysiologist, one of the founders of the phytohormonal theory, creator of tropism hormonal theory and one of the founders of phytohormone doctrine. He was the first to formulate the notion "hormone". Plant hormone research had become rapidly developing study within modern plant physiology since Kholodny. He has founded the physiological polifunctionality of plant hormone auxin, role of hormone synthesis and transport. The main phytohormonal research activities in Ukraine concern phytohormonal regulation of growth and development processes in plants both on the cell and whole plant level, adaptive role of growth regulators and phytohormones as well as agricultural use of synthetic and natural growth regulator substances.

The object of the Symposium is to promote the progress of the study of plant growth substances at the international level. A plant growth regulator is an organic compounds, either natural or synthetic, that while in low concentration modify or control one or more specific physiological processes in a plant. Entire life of plants from fertilization of the egg cell up to the senescence and death is controlled by phytohormones, they also play an important role in plant responses to environmental factors and in forming plant tolerance to extreme conditions. Virtually every aspect of plant growth and development stays under hormonal control to some degree. A single hormone can regulate considerable diverse arrays of cellular and developmental processes, while at the same time multiple hormones often affects a single process. Well-studied examples include the promotion of fruit ripening by ethylene, regulation of the cell cycle by auxin and cytokinin and the maintenance of seed dormancy by ABA. Historically, the effects of each hormone have been defined largely by the application of exogenous hormone. More recently, the isolation of hormone biosynthetic and response mutants has provided new powerful tools for visualizing a clearer picture of the roles of various phytohormones in plant growth and development.

The study of hormonal regulation of plants is one of "hot points" of world biochemistry, physiology, and plant molecular biology. The most crucial problem of modern plant hormone research is a discovery of primary effects evoked by phytohormones in cells leading to modulation of plant growth and development. While great strides have been made in understanding of the molecular basis of phytohormone action in recent years, many fundamental questions remain to be solved. Receptors and other upstream signaling components remain to be identified for the majority of the phytohormones. Equally important are the elucidation of hormonal networks and the integration of these networks within the morphogenetic program, and thus our understanding of hormone action can be placed in a developmental context.

It's a great pleasure for me to mention a contribution of Institute of Bioorganic Chemistry and Petroleum chemistry researchers in elaboration of plant growth regulator synthesis.

ABSTRACTS
LECTURES

REGULATION OF PLANT GROWTH AND DEVELOPMENT BY CYTOKININ

Tomas Schmülling.
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The recent years have witnessed dramatic progress in understanding metabolism and signalling of the plant hormone cytokinin. This is particularly true for *Arabidopsis* where the major genes of cytokinin biosynthesis, cytokinin breakdown and cytokinin signaling were discovered. These genes were used as tools to establish cytokinin loss-of-function mutants. This was achieved either by constitutive or tissue-specific overexpression of different cytokinin oxidase/dehydrogenase (*CKX*) genes or by isolating and combining genetically knockout alleles of the three receptor genes. This presentation will mainly address the question what we have learned from the resulting mutants - which have either a lowered cytokinin content or a reduced cytokinin signaling - about the functions of cytokinins in regulating plant development. A number of cytokinin-dependent processes during vegetative development of roots and shoots, as well as during generative development, were identified. Potential biotechnological applications will be discussed. In addition, some analytical data of the Arabidopsis *CKX* and *AHK* gene families and progress in finding links between the cytokinin signaling system and the rest of the *Arabidopsis* genome and proteome will be addressed.

PHOSPHOLIPASES AND PHOSPHATIDIC ACID IN HORMONAL SIGNALING AND STRESS RESPONSES

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Cell membranes are the initial and focal points of stimulus perception and signaling messenger production. Signal transduction, vesicular trafficking, and many other critical cellular functions are initiated by the assembly of cytosolic protein complexes to specific sites in cellular membranes. Binding to specific lipid ligands are required for the recruitment and/or regulation of the signaling and meatbolic complexes. In recent years, phosphatidic acid (PA), the simplest membrane phospholipid and also a central intermediate of glycerolipid metabolism, has emerged as a class of pivotal lipid mediators in various cellular processes. The effects of PA have been linked to signaling and production of phytohormones and to plant growth, development, and responses to abiotic and biotic stresses. The modes of PA action are multifaceted and include membrane tethering, direct modulation of enzymatic activity, and effects on membrane structures and metabolism. Signaling PA can be produced by multiple enzymes, and the activation of specific enzymes regulates the timing, location, and molecular species of PA. Phospholipase D (PLD) is one major family of enzymes that produce PA. All the PLDs characterized display distinguishable regulatory properties, and the functions of specific PLDs and PA have been linked to plant responses to various stresses, including water deficits, freezing, salinity, and nutrient deficiency. Recent results have provided insights into different mechanisms by which different PLDs and PA mediate hormonal and stress responses.

SENSING CHANGES IN AUXIN CONCENTRATION

Richard Napier
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Auxin has been recognised as an important plant hormone for many decades. Its role in many diverse responses attracts an ever increasing number of scientists. As a community we now have detailed insights into how auxin signalling is initiated. The mechanisms of how auxin is recognised by receptors (how the plant measures its auxin concentration) will be described, drawing on the structural detail provided by crystallography of both TIR1 and ABP1. Questions of sensitivity will be discussed. In the second part of the presentation I will present the case for developing auxin biosensors which can be quantitative, real-time and accessible for living plant tissues. Neither of the receptor proteins has proved adaptable as a tool for reporting quantitative fluxes in auxin concentrations. I will review methods which have been used to assess auxin concentrations in plant tissues, genetic reporters, immunology and mass spectrometry to name a few. Only the last is reliably quantitative and this requires tissue homogenisation. There is a need for further development of auxin biosensor technologies and the prospects will be reviewed.

REGULATION OF CYTOKININ ACTION IN PLANTS: BIOSYNTHESIS AND ACTIVATION

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Cytokinin (CK) plays a crucial role in various phases of plant growth and development and the concentration is finely controlled by internal and external environmental factors such as phytohormones and nitrogen sources. CK in plants is first synthesized as isopentenyladenine (iP) nucleoside phosphate by adenosine phosphate-isopentenyltransferase (IPT) and then hydroxylated to trans-zeatin (tZ) nucleoside phosphate by a cytochrome P450 monooxygenase, CYP735A. The expression of a subset of IPT genes is tightly regulated by nitrogen sources, such as nitrate, and that of CYP735A is induced by cytokinin and repressed by auxin. Our recent study with a rice mutant, *log*, an activation step of CK is catalyzed by LOG, a cytokinin-specific phosphoribohydrolase, which converts the CK-nucleotide to the biologically active free-base form. Spatial expression patterns of the genes indicate that CK is locally synthesized and activated at various sites where it is needed. However, occurrence of CK in xylem and phloem, and our recent study on *cyp735a1/a2* double knock out mutant strongly support that tZ, which is translocated from root to shoot via xylem, plays an important role for shoot normal development. We will outline the recent progress of study on metabolic pathway of CK and discuss the regulatory system in plants.

References:
Takei, K. et al. (2001) *J. Biol. Chem*. 276: 26405-26410.; Kasahara, H. et al. (2004) *J. Biol. Chem*. 279: 14049-14054.; Takei, K. et al. (2004) *Plant Cell Physiol.* 45: 1053-1062.; Takei, K. et al. (2004) *J. Biol. Chem*. 279: 41866-41872.; Sakakibara, H. et al. (2005). *Proc. Natl. Acad. Sci. U.S.A.* 102: 9972-9977.; Kurakawa, T. et al. (2007) *Nature* 445: 652-655.; Sakakibara, H. (2006) *Annu. Rev. Plant Biol.* 57: 431-449.; Sakakibara, H. et al. (2006) *Trends in Plant Science* 11: 440-448.

LIGAND-BINDING PROPERTIES AND ACTIVATION OF CYTOKININ RECEPTORS

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Cytokinins were discovered in the F. Skoog's laboratory (USA) more that 50 years ago, but only recently the important progress has been made in elucidation of the molecular mechanism of their action. At present, three histidine kinases are considered as cytokinin receptors in Arabidopsis: AHK2, AHK3 and CRE1/AHK4. In order to study the properties of cytokinin receptors in more detail, we have used the model system based on transgenic bacteria expressing individual cytokinin receptors (Suzuki et al., Plant Cell Physiol., 2001). It was shown previously, that the receptors CRE1/AHK4 and AHK3 expressed in bacteria were responsive to cytokinin, i.e. retained their functional properties (Yamada et al., Plant Cell Physiol., 2001; Spíchal et al., Plant Cell Physiol., 2004). We have developed a live-cell binding assay to characterize and compare the hormone-binding ability of the receptors (Romanov et al., An. Biochem., 2005; J. Exp. Bot., 2006). By using highly labeled ^{3}H-zeatin, it was shown that bacteria transformed with *CRE1/AHK4* or *AHK3* acquired the ability to bind specifically cytokinins but not related non-hormonal compounds or other phytohormones. Some important characteristics of cytokinin-receptor interaction (affinity constants, ligand specificity of binding, pH-dependence, etc.) will be presented in the lecture. We found a clear difference in binding specificity between two types of receptors, CRE1/AHK4 and AHK3, regarding their affinity towards the iP-type cytokinins: CRE1/AHK4 binds iP-cytokinins much more strongly than does AHK3. Both cytokinin receptors strongly bind *trans*-zeatin. Thus it appears that AHK3 is tuned to respond mainly to a long-distance signal representing root-derived *trans*-zeatin.

It now becomes clearer how the cytokinins activate the appropriate receptors and what happens thereafter. By analogy with the bacterial sensor histidine kinases, it was suggested and strongly confirmed (Kakimoto, Annu. Rev. Plant Biol., 2003; Hwang & Sakakibara, Physiol. Plant., 2006) that receptors are activated by autophosphorylation and then the signal is transduced via phosphotransfer proteins to a set of primary response genes. Our data show that other factors might be important for cytokinin signal transduction as well, in particular phospholipase(s) D. As regards cytokinin primary response genes, they were shown to consist of a small portion (less than 1%) of the plant genome and are enriched with genes participating in transcriptional regulation. But the secondary transcriptional effects which occur a bit later concern a rather large proportion of the genome (thousands of genes, Brenner et al., Plant J., 2005). To sum up, we can conclude that:

Cytokinin receptors differ in functional (ligand-binding) properties. This could ensure tight signal communication between aerial and underground parts of plant, thus maintaining the integrity of plant organism;

Cytokinin signal transduction probably involves not only elements of two-component system of bacterial type, but also some elements of eukaryotic signaling, particularly phospholipase D;

Cytokinins seem to change overall gene expression via transcriptional cascades, firstly activating a small number of primary response genes which encode regulatory proteins (transcription factors).Supported by grants RBFR (Russia) NN 07-04-91211-ЯФ and 07-04-00331

METABOLIC CONVERSION OF CYTOKININS

[1]Frébort I., [1]Galuszka P., [1]Pospíšilová H., [2]Frébortová J., [1]Kopečný D., [1]Šebela M.
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Cytokinins are plant hormones that contribute to regulation of a variety of developmental processes including apical dominance, flower and fruit development, leaf senescence, and seed germination. Cytokinins bind to cell surface receptors and initiate a signal transduction cascade leading to activation of specific genes. Structural features including the nature of the side chain that is attached to the adenine moiety, conjugation with sugars and phosphorylation greatly affect the biological activity of cytokinins.
Cytokinins are selectively inactivated by oxidative cleavage of their side chain by cytokinin dehydrogenase (CKX, EC 1.5.99.12), a flavoprotein containing covalently bound redox cofactor FAD that is presumed to recycle during the catalytic reaction via quinones derived from oxidation of plant phenolics. Genome-wide studies revealed that in higher plants, CKX proteins are encoded by small gene families with a varying number of members. Study of the seven members of the *Arabidopsis thaliana* CKX family shows that secreted enzymes AtCKX2, AtCKX4, AtCKX5 and AtCKX6 regulate the level of cytokinins in apoplast, cleaving preferably free cytokinin bases. AtCKX1 that may recycle components of cytokinin molecules in vacuoles shows specificity to cytokinin ribosides and cytokinin N^9-glucosides. Similarly, AtCKX7, showing also high preference for cytokinin N^9-glucosides, may perform this function in cytoplasm. The physiological meaning for such a preference is unclear so far, because cytokinin N^9-glucosides were found inactive in many biotests. Another vacuolar enzyme, AtCKX3, degrades preferentially cytokinin nucleotides. Substrate specificity of CKX enzymes seems to be predetermined by the nature of a single amino acid residue positioned near the entrance to the substrate channel that interacts with N^9 atom of the cytokinin. Neither AtCKX enzyme exhibits specificity towards aromatic cytokinins that are very weak substrates. Further investigation on cytokinin degradation showed that yeast adenine deaminase (EC 3.5.4.2) from *Schizosaccharomyces pombe* hydrolyzes cytokinins at significant rates (including the aromatic ones), but this activity has not been detected yet with any plant enzyme.

CYTOKININS: THEIR UNEXPECTED ROLE AS PCD INDUCERS

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High levels of cytokinins induce programmed cell death (PCD) both in animals and in plants. A high dosage of the cytokinin BA induces PCD in proliferating plant cell cultures as demonstrated by DNA laddering and chromatin condensation and the release of cytochrome *c* from mitochondria into the cytosol. Usually, the release of cytochrome *c* occurres prior to the cleavage of DNA, suggesting a temporal sequence of apoptotic steps.
Apparently, high levels of BA induce PCD by accelerating senescence. In fact, the process is relatively slow, taking place in 4-5 days, and cell death and DNA fragmentation is preceded in *Arabidopsis* by early expression of *SAG12* , a gene coding for a cysteine protease specifically associated with leaf and cell culture senescence. During cell death induced by cytokinin in *Medicago truncatula* cell culture, the reticular arrangement of mitochondria, characteristic of healthy growing cells, disintegrates rapidly. MtSAG (an ortologue of *Arabidopsis SAG12*) transcript levels increase and giant mitochondria, usually associated with high levels of cell death, are detected together with an increase of release of cytochrome *c*. Hence mitochondria appear to play a central role in this pathway of cell death.
In order to identify signalling intermediates of cytokinin-induced PCD, the role of nitric oxide (NO), a key signalling molecule, was analysed in *Arabidopsis* suspension cells. We observed that BA induces NO synthesis in a dose dependent manner. NO appears to be produced via a NOS enzyme since both its level and cellular effects were strongly reduced by pre-treatment with a NOS inhibitor and a NO scavenger. More interesting, in cells incubated with BA in the presence of NOS inhibitor, cell death was significantly reduced and cell growth inhibition was attenuated, suggesting for NO an early signalling role in this pathway of PCD induced by BA.
In BA-treated cells, mitochondrial functionality is altered via inhibition of respiration. It was not unexpected as we have previously showed that NO affects mitochondrial functionality in plant cells reducing total cell respiration by inhibiting the cytochrome pathway. But this inhibition can be prevented by addition of NO scavenger or NOS inhibitors implying that NO acts at the mitochondrial level.
Preliminary results on the physiological role of high cytochinin concentration in plants will also be discussed.

CONTROL OF *AHP6*, A CENTRAL PLAYER OF VASCULAR DEVELOPMENT IN THE *ARABIDOPSIS* ROOT

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During vascular development in the *Arabidopsis* root, cytokinins promote pluripotent cell as well as phloem identity and inhibit protoxylem cell identity. Protoxylem cell fate is dependent on the localised inhibition of cytokinin signalling by AHP6, a pseudo-phosphotransfer protein that acts to inhibit the phosphorelay associated with cytokinin signalling. *AHP6* is expressed specifically in both protoxylem cell files. Conversely, cytokinin signalling negatively regulates the spatial domain of *AHP6* expression. Consequently, a negative regulatory feed-back loop operates where cytokinin signalling counteracts expression of its inhibitor facilitating protoxylem formation. The identity of either the negative regulatory (cytokinin mediated) or promotive factors which converge on *AHP6* is unknown. To identify and characterize upstream factors controlling *AHP6*, a forward genetic screen was performed to identify altered expression patterns of *pAHP6::GFP* within an EMS mutagenized line. A set of novel mutants was identified and the phenotypic description of these genetically interacting loci will be presented. Further functional and molecular characterization of those loci can reveal the basic genetic mechanisms underlying vascular development.

CYTOKININ CONTENT IN SHOOTS IN RESPONSE TO ROOT TREATMENT. IS CYTOKININ TRANSPORT INVOLVED IN ROOT/SHOOT SIGNALING?

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Plants have to coordinate numerous processes taking place in their different parts. Exchanges of hormonal signals between shoots and roots are believed to be implicated in the control of their coordinated growth (Jackson, 1993). But it remains unclear if cytokinin signals always come from treated roots. In our research we applied different treatments resulting in activation of root growth: partial derooting of wheat plants (cv. Bezenchukskaya 139) and 10-fold dilution of their Hoagland-Arnon nutrient solution. Excision of 4 out 5 seminal roots decreased cytokinin content in xylem sap determined by means of immunoassay. Since transpiration did not change significantly delivery of cytokinins remained the same as in intact plants. Restoration of cytokinin flow to shoot was observed only one day after the treatment. Unexpectedly cytokinin content in shoots was higher in partially derooted plants than in intact plants already in 1 hour. Which mechanisms of cytokinin accumulation may be involved? It may be a reduced rate of cytokinin (CK) decay first of all. Activity of cytokinin oxidase (CKO) was determined as the rate of degradation of isopentenyladenine after addition of proteins extracted from leaves to incubation medium. Expression of the gene coding for CKO was estimated by means of RT-PCR. A decline in activity of the enzyme and CKO gene expression were observed. Accumulation of CK due to a reduced rate of their decay contributed to maintaining shoot growth of partially derooted plants at the level of intact plants both directly and through their effect on photosynthesis.

Deficit in mineral nutrition led to no changes in cytokinins content in xylem sap and their delivery from roots to shoots within one day after the treatment. However by this time cytokinin content in shoots decreased two times. Unlike experiments with partial derooting dilution of the nutrient solution resulted in accumulation of ABA in leaves as compared to control plants. The source of ABA accumulation may be the bound ABA transported from roots, which elevated level was registered in xylem sap of plants grown on diluted nutrient solution. According to literature data ABA may influence metabolism of cytokinins (Brugiere et al., 2003). We measured activity of CKO and expression of CKO gene in shoots and observed an increase in both enzyme activity and expression of its gene. Consequently cytokinin signal may be generated in shoot itself under the influence of ABA on the rate of cytokinin decay. It is obvious that a study of implication of hormones in shoot/root interaction demands analysis of not only the transport of hormones from roots to shoots, but also of the mechanisms generating hormonal signal in the target tissue.

Thus under different influences cytokinins alongside with ABA are involved in root signaling and play a great role in coordination of roots and shoot growth. Mechanisms of signaling may be different depending on environment and internal factors.

The work was supported by RFBR (grant 05-04-50824)

THE MEKK1-MKK1/2-MPK4/6 MAPK PATHWAY COORDINATES STRESS RESPONSES WITH REACTIVE OXYGEN SPECIES AND HORMONE SIGNALING

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We have shown that the *Arabidopsis* MAPK kinase MKK2 is involved in mediating abiotic stress responses (Teige et al., 2004). In agreement with such a function, transcriptome analysis of constitutively active MKK2-EE plants showed altered expression of genes induced by abiotic and biotic stresses, including enhanced levels of genes encoding enzymes of ethylene (ET) and jasmonic acid (JA) synthesis. Our recent analysis showed that in contrast to wild type, MKK2-EE plants are comropmised in JA and SA accumulation upon *P. syringae* infection, indicating that MKK2 is involved in regulating hormone levels in response to pathogens (Brader et al., 2007). MKK2-EE plants were found to be more resistant to infection by *P. syringae* and *Erwinia carotivora*, but less resistant to the fungal necrotroph *Alternaria brassicicola*. Various experimental evidence suggests that MEKK1 is the upstream activator of the MKK2-MPK4/MPK6 module and its downstream targets. A genetic analysis of *MEKK1, MKK2, MPK4* and *MPK6* revealed that *MEKK1*- and *MPK4-,* but not *MKK2* or *MPK6*-deficient plants accumulate reactive oxygen species and exhibit a lethal phenotype that is correlated with misregulation of ROS biosynthesis and detoxification genes (Nakagami et al., 2006). These and other data suggest that the MAPK module MEKK1-MKK1/2-MPK4/6 is involved in mediating abiotic and biotic stress responses.

AUXIN RESPONSE NETWORKS IN EMBRYONIC ROOT FORMATION

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The root meristem is first initiated in the developing embryo, and requires specification of an extra-embryonic suspensor cell as hypophysis, the quiescent centre precursor cell. Specification of the hypophysis is controlled by the Auxin Response transcription Factor (ARF) MONOPTEROS (MP), and its inhibitor IAA12/BODENLOS. We have found previously that MP acts in a small group of cells adjacent to the future hypophysis, which implicates cell-cell communication through secondary signals in hypophysis specification. Auxin itself is such a signal, since MP controls PIN1-dependent auxin transport from the embryo to the upper suspensor cell, where it elicits a second auxin response. However, auxin accumulation alone seems insufficient for hypophysis specification. In order to identify additional signalling pathways, we have used microarrays to isolate a number of transcription factor genes that are immediate targets of MP. We will present functional analysis of these target genes in root initiation. Furthermore, we have used a reverse genetics approach to identify additional ARF and AUX/IAA proteins in hypophysis specification and found that in addition to hypophysis cell fate, many cell fates in the early embryo are controlled by ARF transcription factors. We will also present our progress on the systematic dissection of auxin responses in embryo development.

ROLE OF THE AUXIN-BINDING PROTEIN 1 IN THE CONTROL OF ROOT GROWTH

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Auxin Binding Protein 1, ABP1, was identified over 30 years ago by its capacity to bind auxin. It was rapidly established, via the accumulation of a large range of electrophysiological data, that ABP1 is involved in the control of very early auxin responses, including activation or deactivation of ion channels (K^+, anions) or transporters (proton pump ATPase) in an auxin dose dependent manner[1]. More recently, ABP1 was shown to be essential for early embryo development[2], reflecting a critical importance to the plant. To further investigate the role of ABP1, we have generated conditional knock-down for ABP1 using cellular immunisation. This approach is based on the *in vivo* expression of recombinant immunoglobulin fragments termed scFv (single chain Fragment variable) consisting of the heavy and light chain variable domains of an antibody linked by a flexible peptide. We have made use of well characterised monoclonal antibodies to construct such scFv fragments and target inactivation of ABP1. This approach was used first to conditionaly impair ABP1 function in tobacco BY2 cells[3], thus demonstrating that ABP1 is involved in the control of the cell cycle in these cells, most likely by mediating auxin action. This approach was then tranferred to the whole plant and conditional knock-down were generated in Arabidopsis. Inactivation of ABP1 provokes severe growth and developmental defects amongst which a strong inhibition of root growth.

1. Napier, R.M., David, K.M. and Perrot-Rechenmann, C. (2002) A short history of auxin-binding proteins. *Plant Mol Biol*, 49, 339-348.
2. Chen, J.G., Ullah, H., Young, J.C., Sussman, M.R. and Jones, A.M. (2001b) ABP1 is required for organized cell elongation and division in Arabidopsis embryogenesis. *Genes Dev*, 15, 902-911.
3. David K.M., Couch D., Braun N., Brown S., Grosclaude J., Perrot-Rechenmann C. (2007) The auxin-binding protein 1 is essential for the control of the cell cycle. *Plant J.*, 50, 197-206.

BRASSINOSTEROIDS: A NEW TYPE OF SIGNALING MOLECULES AND BASIS FOR DEVELOPMENT OF ECOLOGICALLY FRIENDLY AGROCHEMICALS

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Recognition of brassinosteroids (BS) as a new class of plant hormones was an epoch-making discovery of XX century, because to the previously known role of steroids as hormones of humans, animals, insects and fungi their hormonal functioning in plants has been added [1,2]. This brought a new understanding of steroids as versatile bio-regulators characteristic for all living creatures. An important physiological effect of new plant hormones, when applied exogenously to growing plants, is their capability to stimulate plant growth and development. That is why their application in agriculture was considered to be promising from the beginning of BS studies and later found its realization [3].

After the discovery of brassinolide, the first member of the series, progress in brassinosteroid research has been extremely rapid, and less than twenty years were necessary to start agricultural use of BS. During this period, extensive fundamental and applied studies were carried out including elaboration of economically feasible approaches to BS synthesis, laboratory and field-scale biological trials with different crops, toxicological studies, solving the problems on industrial-scale production and official status of new agrochemicals, etc. For none of the other plant hormones, although studied for a much longer time, there has been similar development.

All the data on BS activities in plants, the discovery of genes that are specifically expressed by BS, and the identification of BS-receptor make sure that BS are real plant hormones. During recent years, the molecular genetic methodology and use of special mutants of *Arabidopsis* brought real break-through in the mechanistic studies of BS-action. The findings in disclosure of the mechanism stimulate further efforts directing to the localization of the effect of BS in the chain of signaling events in the plant cell. One of the indicators on the promising area is the data on the genetically-determined involvement of BS in the light-regulated plant development. Our study on the effect of BS on the hormonal balance in light-dependent development of wild type *Arabidopsis* and its mutants defective in genes encoding synthesis of some photoreceptors showed clear relationships between the responses mediated by these photoreceptors and action of BS. The results suggest an important function of BS in light and hormone signaling cross talk and give a new confirmation of their central role among other phytohormones.

Although a number of problems have been solved, there are still those playing a critical role for further development of the area and demanding deeper investigation. Among them: further development of synthetic and analytical methodology and search for structural species-specificity in natural distribution of BS, further mechanistic studies and localization of BS-effect in cell signaling, study on structural species-specificity of plant physiological response to exogenous BS in connection with the biosynthesis and metabolism of the endogenous BS. All these are very important for higher reliability and predictability of agricultural use of BS. Our recent results in the area will be discussed.

1. Khripach V. A., Zhabinskii V. N., de Groot A. *Brassinosteroids. A new class of plant hormones*; Academic Press: San Diego, **1999**. 2. Adam G., Schmidt J., Schneider B. *Progress in the Chemistry of Organic Natural Products* **1999,** *78*, 1-46.3. Khripach V. A., Zhabinskii V. N., Khripach N. B. In *Brassinosteroids;* S.Hayat; A.Ahmad Eds.; Kluwer Academic Publishers, **2003**; pp. 189-230.

REGULATION OF BRASSINOSTEROID SIGNALING

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Brassinosteroids are a unique class of plant polyhydroxysteroids critical for normal plant growth and development. Arabidopsis mutants defective in brassinosteroid biosynthesis or signaling exhibit a characteristic set of growth/developmental defects including a dwarf stature, reduced male fertility, delayed flowering, abnormal vascular differentiation, and aberrant skotomorphogenesis. Genetic and biochemical studies in the past decade have revealed a linear brassinosteroid signal transduction pathway that involves two cell surface receptor kinases, a GSK3-like kinase, and two GSK3 substrates that can directly bind DNA to regulate gene expression. I will summarize the current knowledge of this phosphorylation-mediated signaling pathway. In addition, I will discuss a potential regulatory mechanism for restricting the GSK3-like kinase activity in response to activation of two receptor kinases and potential roles of two families of helix-loop-helix proteins in brassinosteroid signaling.

ABSCISIC ACID AND BRASSINOSTEROIDS HAVE AN OPPOSITE EFFECT ON THE MODULATION OF THE PROTON PUMPING AND THE ANION CHANNEL ACTIVITY AT THE PLASMA MEMBRANE OF *ARABIDOPSIS THALIANA SUSPENSION C*ELLS: VACUOLAR CALCIUM DEPENDENCY ?

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The plant growth regulators abscisic acid (ABA) and 28-homobrassinolide (HBL) play key roles in the control of plant development and cell volume by regulating ion channel activities and water exchanges across the plasma membrane (PM).
In *Arabidopsis thaliana* suspension cells, our results clearly show that both ABA and HBL had opposite effect on the modulation of the proton pump and anion channel activity. These modulations were associated with the control of the PM electrical gradient magnitude involved in phytohormones signaling pathways.
Using experiments employing combined voltage clamping and continuous measurement of extracellular pH during PM phytohormone signaling on cells where physiological wall functions are maintained, we demonstrate that HBL induced both medium acidification (ΔpH $\approx$ 0.45 units in less than 10 min) and PM hyperpolarization (ΔEm $\approx$ -12 mV), whereas ABA simultaneously induced rapid alkalinization of the medium (ΔpH $\approx$ 0.06 units) and PM depolarization (ΔEm $\approx$ 6 mV). These data revealed that the PM H^+-ATPase is activated by HBL (Zhang et al. 2005), but inhibited by ABA (Brault et al. 2004) in *A. thaliana* suspension cells. Upon ABA treatment, we observed an increase in the anion current (anion efflux) in suspension cells (ΔI $\approx$ 62%). This increase is abolished by a subsequent addition of the anion channel inhibitor 9-AC (ΔI $\approx$ 17%) or strongly reduced when ABA was added in the presence of 9-AC. In opposite manner, we observed HBL treatment decrease anion current in suspension cells (ΔI $\approx$ 70%) during the PM hyperpolarization. Therefore, anion channels may also be good candidates, in addition to proton pumps for the controls of PM potential during the responses to phytohormones signaling (Zalejski et al. 2006). These effects could be prevent by the targeted interruption of the signaling pathway either at the level of the PM Ca^{2+} channels or (and) the tonoplast level of the cADPR, IP_3 and ryanodine-induced intracellular calcium increase.

1. Zhang Z, Ramirez J, Reboutier D, Brault M, Trouverie J, Pennarun AM, Amiar Z, Biligui B, Galagovsky L, Rona JP. 2005. Brassinosteroids Regulate Plasma Membrane Anion Channels in Addition to Proton Pumps During Expansion of *Arabidopsis thaliana* Cells. Plant Cell Physiol. 46 (9) : 1494-1504.
2. Brault M, Amiar Z, Pennarun, AM, Monestiez, M, Zhang, Z, Cornel, D, Dellis, O, Knight, H, Bouteau, F. and Rona, JP. (2004) Plasma membrane depolarization induced by abscisic acid in A. thaliana suspension cells involves reduction of proton pumping in addition to anion channel activation, which are both Ca2+ dependent. Plant Physiol. 135:231-243.
3. Zalejski C., Paradis S., Maldiney R., Habricot Y., Miginiac M., Rona JP., Jeannette E. (2006) Induction of ABA-regulated gene expression by diacylglycerol pyrophosphate involves Ca2+ and anion currents in Arabidopsis thaliana suspension cells. Plant Physiol. 141:1555-62

THE INFLUENCE OF *CYP11A1* GENE EXPRESSION ON THE REGULATORY SYSTEM AND PHENOTYPE OF TRANSGENIC TOBACCO PLANTS

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Close similarity of steroid biosynthesis in animal and plant cells, where cytochromes P450 take a considerable role, should be noted. The main difference between steroidogenic systems in plants and animals is the absence of cytochrome P450scc in plants. This enzyme catalyzes the conversion of cholesterol into pregnenolone – the precursor of all steroid hormones in animals. This process occurs only in animal mitochondria and proceeds with participation of the other two proteins of electron transfer chain – adrenodoxin and adrenodoxin reductase that homologes are also found in plant mitochondria. All above mentioned give us the possibility to suppose that cytochrome P450scc can display its catalytic activity in plant cells.

The presence of animal steroid hormones in a range of higher plants and their involving in growth and development regulation has been established recently. Moreover it has been shown that progesterone at low concentrations can promote plant growth and suppresses it at higher concentrations.

With the aim to study the synthesis of mammalian hormones and its possible influence on the plant growth and development the transgenic *Nicotiana tabacum sv. Petit Havana SR1* plants carrying cDNA of *CYP11A1* gene encoding this protein have been created. Transformation vector *pGBP450f* was constructed on a basis of *pGreen0229* binary plasmid where the cDNA of *CYP11A1* was under the *CAMV 35S* promoter. Southern blot and RT-PCR analyses confirmed the presence and the expression of *CYP11A1* in transgenic plants obtained. The pregnenolone in the steroid fraction has been identified by gas chromatography mass spectrometry method. Its further conversion into progesterone was verified using the enzyme immunoassay method.
Transgenic plants exhibit an enhanced growth and development rate as compared to the wild type plants. The earlier flowering of all transgenic lines has been observed as well.
The protein and carbohydrates contents in leaves and seeds of transgenic plants exceed noticeably those in control plants. At the same time the difference from line to line has also been observed.
The results obtained indicate that *CYP11A1* expression in transgenic tobacco plants makes considerable alterations in their regulatory system that is accompanied by changes in phenotype.

THE ROLE OF BRASSINOSTEROIDS IN TRANSDUCTION OF GREEN LIGHT SIGNALS

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It was assumed that brassinosteroids (BRs), possibly, participate in transduction of light signal (T. Takematsu, 1988). It is known that biosynthesis and mechanism of BRs inactivation as well as response reaction to BRs are under red light (phytochromes) and blue light receptors (cryptochromes) control. Despite numerous physiological studies of this question, it is little known about the participation of BRs in transduction of light signal. The interaction between green light and brassinosteroids have not been researched yet. The reception of green light signals has not been studied enough. In 1995 C. Lin presupposed that cryptochrome was one of probable green light photoreceptors.

We studied the role of brassinosteroids (brassinolide, epibrassinolide and homobrassinolide) in transduction of green light signal. The universal plant model to study regulatory function of BRs in plant morphogenesis at green light is *Arabidopsis thaliana*. Plants of *A. thaliana* ecotypes Landsberg *erecta* (L*er*) and Columbia (Col) as well as mutants *hy4* (mutated in cryptochrome) and *det2* (disturbed synthesis of brassinolide) were used.

The study of growth of etiolated seedlings of *A. thaliana* showed that the length of hypocotyls and areas of cotyledons of *hy4* were significantly less than those of wild type L*er*. The mutant *det2* differed from wild type Col having shorter hypocotyl and also bigger areas of cotyledons. The reaction of ecotypes to BRs in the darkness differed from mutants' one and manifested in inhibition of Col, L*er* and *hy4*'s length of hypocotyls and roots and also in stimulation of *det2* axes organs growth. The biggest bioactivity with respect to Arabidopsis seedlings was noted for brassinolide, than bioactivity of epibrassinolide for Col and *det2* and homobrassinolide for L*er* and *hy4* came.

Deetiolation of Arabidopsis seedlings for 15 minutes with green light (λ = 543 nm, 29 $\mu mol/m^2s$) induced the processes analogous to the action of exogenous BRs. The green light activated photomorphogenesis in Arabidopsis seedlings of wild type and of mutants with disturbed synthesis of brassinosteroids and cryptochrome. The inhibiting influence of green light on *det2* hypocotyl growth was displaced by exogenous BRs. The analogous effect was observed for wild type seedlings (Col) while using epibrassinolide and brassinolide in concentration 10^{-8} M. Simultaneous influence of brassinosteroids (brassinolide, epibrassinolide and homobrassinolide) and green light resulted in significant increase of Col and *det2* cotyledons areas comparing to their separated influence.

Simultaneous influence of brassinosteroids and green light on L*er* and *hy4* Arabidopsis morphogenesis displayed addition of effects in the growth of hypocotyl and cotyledon areas. Thus, it may be suggested that BRs can be involved in transduction of green light signals in Arabidopsis seedlings morphogenesis as alternative messengers.

EFFECTS OF PLANT GROWTH SUBSTANCES ON MEMBRANE PHOSPHOLIPID METABOLISM

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We have studied the changes in phospholipid metabolism that are caused when pea shoot elongation is altered by growth substances. Both gibberellic acid (GA_3) and indole acetic acid (IAA) were used but the latter gave larger, and more reproducible, effects. Altered stem growth was characterised by several changes in lipid metabolism, of which altered phosphatidylcholine synthesis was noticeable.
Phosphatidylcholine is the main non-chloroplast membrane lipid in plants and is synthesised mainly by the CDP-base pathway. Of the enzymes involved in this pathway, the first (choline kinase) and the middle (cholinephosphate cytidylyltransferase: CPCT) have been claimed to exert regulation. We have purified both enzymes and isolated cDNAs for them. Studies on the regulation of phosphatidylcholine formation in response to IAA showed that CPCT was the most important enzyme for control of flux. Mechanisms for its regulation have been examined and will be discussed.

ROLE OF PHOSPHOLIPASES C AND D IN THE PHYTOHORMONES ACTION AND STRESS RESPONSE OF THE PLANT MERISTEM AND MATURE CELLS METABOLISM. *IN VIVO* INVESTIGATION

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Plant hormones plays important roles in many cellular processes including seed development, dormancy, germination, growth, and environmental stresses, such as drought, cold and salinity, but the molecular mechanism of their action is far from being understood. Individual plant cells can both directly sense and respond to the extra cellular condition. It is well known that phospholipids are key regulators of a plant cells metabolism. Many extra cellular signals are perceived by plasma membrane receptors and converted into intracellular responses via phospholipases C and D (PI-PLC and PLD). The effect of different stress and phytohormones on the *in vivo* activity PI-PLC and PLD of the mature plant tissues as well as plant meristems were studied. Detached leaves and roots of corn were placed in flasks containing [^{33}P]orthophosphate for 16 h at 25°C. It was shown that in maize leaves ^{33}P incorporated into phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid (PtdOH) and polyphosphoinositides . For the analyses PI-PLC activity the level phosphatidyl inositol 4,5-bisphosphate (PIP2), phosphatidyl inositol 4-phosphate (PI(4)P),as well as inositol (1,4,5) - trisphosphate was investigated. PLD activity was determined as the level incorporation ^{33}P in the phosphatidylbutanol (PBut) *in vivo.* Our results strongly suggest that stress conditions and Phytohormones can activate PLC and PLD of the mature plant tissues as well as plant meristems. Theses phospholipases closely cooperates in the signaling network and involved other lipid-signaling enzymes, first of all phosphatidylinositol kinases and phosphatases.

This work was supported by the grants: INTAS N602/2001; NASA-UNTC № 07(R) 2003; Fundamental Research fund of Ukraine grant № F10/24-2005; № Ф14/253-2007 and NAS of Ukraine № 2.1.10.32-05.

PARTICIPATION PA IN TRANSPORT OF IONS AND BAP SIGNALING

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Phosphatidic acid (PA) takes special place among signal lipids because it is a key link in the membrane lipid metabolism, on the one hand, and it performs functions of lipid signal molecule, on the other hand. Amount of PA in plant cells elevates transiently under the influence of pathogens, ROS, elicitors, ABA and ethylene. Changes in PA level influence physical properties of membranes and their ability to form vesicles. Some data confirms the ability of PA to transport Ca^{2+} ions through membranes of muscular and nervous cells.

We investigated action of PA with different composition (dioleoyl, dipalmitoyl, from egg yolk) on membrane transport of Ca^{2+}, Mg^{2+} and H^+ using plasma and endomembranes vesicles from maize (Zea mays L.) roots and coleoptiles. Also, of this investigation was to study the influence of BAP on the level of PA.
The 4-day-old etiolated maize seedlings were used. The vesicle preparations were obtained by differential centrifugation and subsequent separation in the PEG/DEX aqueous polymer two-phase system (for isolation of plasma membranes) or in the sucrose density gradient (for isolation of endomembrane fraction). The transport of ions were assayed using Ca-sensitive fluorescent probe Indo-1 (loaded into the membrane vesicles) and Ca^{2+}(Mg^{2+})-sensitive probe chlortetracycline (added to the incubation medium). The proton gradient on the tonoplast vesicles was created by activation of vacuolar H^+-ATPase. The reaction was initiated by the addition of ATP. The increasing of H^+ concentration inside vesicles was registered by the potential density probe acridine orange. The preparation of total pure lipids extracts was isolated on method Bligh and Dyer. The fraction of phospholipids was divided TLC a method Vaskovsky V.E.
Three types of PA were used in experiments; PA was allocated from an egg yolk; PA containing two residues of palmitic acid and PA containing two residues of oleic acid. The greatest effect was observed with PA consisted of two residues of oleic fatty acid. PA including in structure two residues of palmitic fatty acid had the least ionophore properties. The ability of PAs to transport calcium ions across membranes is higher at alkaline conditions than at acidic conditions. We had showed that the ionophore properties of PA are not specific. They are capable to function as membranous transmitting agent not only for Ca^{2+}, but also for Mg^{2+}.
The movement of protons along pH gradient was facilitated by PA as well as FCCP, a well-known protonophore. The highest rate of proton membrane gradient dissipation of tonoplast vesicles caused PA, which consists of two residues of oleic acid. PA with two residues of palmitic acid had the least ionophore activities.
Than we analyzed the influence BAP on change level of PA in cells. We show that the action of BAP on coleoptiles or roots induced the increase of quantity PA and change of fatty-acid composition PA. The received results testify that is probable the action BAP activated PLD. We assume, that PA produced by PLD in plant cells.

Work was financial supported by grants RFBR (no.05-04-49619).

THE EFFECT OF PHOSPHATIDIC ACID ON POTATO TUBERS 9-LIPOXYGENASE ACTIVITY

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In plants the activity of lipoxygenases on polyunsaturated fatty acids generates hydroperoxide products, which are known as oxylipines. These compounds take part in plant response on biotic and abiotic stresses. 9-lipoxygenase(9-LO) is membrane associated enzyme. Adsorption stage of enzyme on biologic membrane is the first step in 9-LO activity regulation. Anionogenic lipids, such as phosphatidylserine, cardiolipin, phosphatidylinositol and phosphatidic acid have an effect on 9-LOX.

It was studied as phospholipid phosphatidic acid (PA) influence on oxidation of linoleic acid by 9-LO from *Solanum tuberosum*. Reaction mixture was consisted of 9-LO and mixed micelles of linoleic acid (LA), Lubrol PX and different quality of enzyme effector – PA. It was established that 9-LO had two $pH_{opt.}$ 5.0 and 6.9 in presence of 50mkM phosphatidic acid. In concentration of 50mkM PA had ability to activate 9-LO (13- 15) fold by pH 5.0. In such condition reaction maximum velocity (V_{max}) concurred with lipoxygenase reaction V_{max} without effector by pH 6.9. It was displayed, that 30mkM phospholipid in reaction mixture decreased concentration of half saturation of substrate on 43-67%. Enzyme demonstrated positive cooperation of substrate, reaction curve circumscribed by Hill equation. Hill coefficient value (h) of substrate was 3,34±0.277(pH 6,9) and 5,607±0.889(pH 5.0), that is substrate molecules number increased with change pH to acidic region and they possible to interact with enzyme molecule from 4 to 6. In case of substrate insufficiency 50 and 100mkM LA enzyme demonstrated positive cooperation of PA, it bonded from 4 to 3 effectors' molecules by pH 5,0. Comparative analyze influence of 4-hydroxy-TEMPO displayed, that nonenzymatic process level lower on 20-80% by unphisiological pH in presence of 30-80mkM PA in comparison 9-LO product level without PA in such condition.

According to our data phospholipid phosphatidic acid may direct interact with molecule of 9-LO and increase enzyme activity in condition of substrate limitation and unphysiological pH cellular matrix. PA supports primary product level of 9-LO linoleic acid oxidating via allosteric displacement substrate molecules in enzyme allosteric center, rising enzyme congeniality to substrate and decrease nonenzymatic product level of linoleic acid oxidating. For phospholipase D has been demonstrated analogue ability of PA to allosteric interact with this enzyme. Thus PA can function not only as second messenger in the cell, but this phosphilipid can direct to regulate membrane associated enzymes.

JASMONATE SIGNALLING NETWORK IN *ARABIDOPSIS THALIANA*: CRUCIAL REGULATORY NODES AND NEW PHYSIOLOGICAL SCENARIOS

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Plant development and stress responses are regulated by complex signalling networks that mediate specific and dynamic plant responses upon activation by various types of exogenous and endogenous signals. Jasmonates mediate responses to stress and act like growth inhibitors. The latest work on jasmonates (JAs) signalling has identified new regulatory nodes in the transcriptional network that regulates a number of diverse plant responses to developmental and environmental cues. Therefore the key elements mediating cross-talk between JAs with other signalling pathways that are activated during stress and defence response will be discussed. Most of the work on JAs has been traditionally done in the context of stress; however, new findings implicating JAs in regulating senescence and plant responses to pathogens suggest a common mechanism of JAs action via distinct groups of transcription factors. Moreover, analysis of JA mutants has mostly focused on altered JA-inducible gene expression and defence responses, whereas a detailed analysis of the causes underlying the stunted growth that characterizes some of them has been seldom performed.In my laboratory, we are interested in discovering the cellular components linking plant stress responses to growth processes with the aim to improve seed production, yield and adaptation of plants to their environment. JAs blocks cell cycle progression by inhibiting G1/S and G2/M transitions in tobacco cells. While the molecular mechanisms and downstream responses have not been clarified yet, we are excited by the likelihood that jasmonate is a distress signal, a physiological role of which is to block cell cycle, slowing vegetative growth during defense responses. A summary of the results obtained so far will be presented. In addition, we will report on new physiological scenarios for JAs signalling such as anti-cancer therapy.

SIGNALING SPECIFICITY AND COMPLEXITY IN MAPK CASCADE NETWORKS

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Mitogen-Activated Protein Kinase (MAPK) cascades are pivotal and evolutionarily conserved regulatory modules controlling diverse signal transduction pathways in eukaryotic cells from yeast to human and plants. Plant genomes encode the largest number of putative MAPK cascade genes (e.g., Arabidopsis: more than 100; yeast: 14; human: 34) in all sequenced eukaryotes. Very limited information on MAPK cascade functionality and regulatory mechanisms has surfaced from classical genetic screens in plants. Biochemical, molecular, reverse genetic and transgenic studies have indicated that plant MAPK cascades are important for controlling broad and essential plant processes, including hormone, stress and innate immune signaling in diverse plant species. However, how MAPKs/MPKs and their immediate upstream regulators, MAPKKs/MKKs and MAPKKKs/MTKs, are integrated into the plant signaling networks connecting upstream signals and downstream transcription factors and target genes remains a major challenge in plant biology.
There are many unresolved puzzles regarding the precise physiological roles of MPKs, MKKs and MTKs in plant hormonal, stress, and defense signaling. To circumvent the limitations in classical and reserve genetic analyses, and to examine dynamic and complex actions in MAPK cascade signaling, we have developed MAPK cascade genomic resources and performed extensive cell-autonomous and systematic screens for *Arabidopsis* MAPKs acting downstream of MKKs and MTKs and various signals that activate putative endogenous MPKs in the cell-based transient expression systems. Arabidopsis mesophyll protoplasts have been used to show the conservation of MAPK cascade signaling and similar physiological responses in isolated cells and in intact plants. In the functional genomic analysis of Arabidopsis MAPK cascade signaling, 20 MPK, 10 MKK and 68 putative MTK genes have been analyzed in mesophyll protoplasts to establish a blueprint for potential MAPK cascade functions and connections. The information will serve as the foundation to launch new genome-wide studies linking dynamic and overlapping signal transduction pathways. The MAPK functional genomic project combines global gene expression profiling and bioinformatics tools to dissect distinct and overlapping MAPK cascades with gain-of-function and loss-of-function mutant analyses in response to hormonal, stress, and elicitor signals. The aims are to integrate broad resources and information on plant hormone, stress, and defense signaling and gene regulation to facilitate comprehensive and molecular understanding of the signaling specificity and complexity of the evolutionarily conserved MAPK cascade signaling networks in Arabidopsis.

Funded by the National Science Foundation 2010 program (IOB 0618292)
http://genetics.mgh.harvard.edu/sheenweb/mapk_cascades_nsf.html

DIVERSE STRESS SIGNALS ACTIVATE THE C1 SUBGROUP MAP KINASES

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Mitogen-activated protein kinase (MAPK) cascades play a key role in plant growth and development as well as in biotic and abiotic stress responses. They are classified according to their sequence homology into four major groups (A-D). A large amount of information about MAPKs in groups A and B is available but few data of the C group have been reported. In *Arabidopsis*, C1 subgroup is constituted by to MAPK genes: *AtMPK1* and *AtMPK2*. Gene expression data deposited in public microarray repertories indicate that these genes have very low expression with no relevant changes in their mRNA levels after hormone treatment and stress conditions. Moreover, both genes show very similar patterns of expression. We have studied the activation of AtMPK1/AtMPK2 in response to mechanical injury. In this study, we have used specific antibodies raised against the unique C-termini of AtMPK1 and AtMPK2 to measure the kinase activity by immuno-complex kinase assays. An increase in AtMPK1/2 kinase activity was detected in response to wounding that was blocked by cycloheximide. Jasmonic acid (JA) activated AtMPK1/AtMPK2 in the absence of wounding. Wound and JA-induction of AtMPK1/2 kinase activity was not prevented in the JA-insensitive *coi1* mutant. Other stress signals, such as abscisic acid (ABA) and hydrogen peroxide (H_20_2), activated AtMPK1/2. In addition, we report the isolation of a full-length cDNA for *PsMAPK2*, a C1 subgroup MAP kinase from *Pisum sativum*. The regulation of PsMAPK2 kinase activity in response to diverse stress signals was studied. The results obtained suggest that C1 subgroup MAPKs may have the same functions across species.

ENVIRONMENTAL STRESSES: POLYAMINES AND PLANT RESPONSES

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Plant development and productivity are negatively affected by environmental stresses. The abiotic stresses together represent the primary cause of crop loss worldwide. Plants respond and adapt to the continuous environmental fluctuation with appropriate changes to cope with these stress conditions. There is biological evidence suggesting that plants use general and conserved response mechanism to deal with abiotic stress. Polyamines are low molecular organic cations that are found in a wide range of organisms from bacteria to plants and animals. In plants, polyamines are involved in various physiological events and considered as plant growth regulator. It has been observed that plants significantly accumulate polyamines under biotic and abiotic stresses. However, the physiological significance and the role of polyamines are still under studies. During the last decade, many genes involved in polyamine metabolism have been identified from several species and their expression profiles in relation to the developmental stages have been analysed.
The *Mesembryanthemum crystallinum* L. (common name: ice plant), in native habitat, germinates and establishes in short, cool and moist winters, followed by dry summer coupled with increasing drought and salinity. The plant shows high adaptability to natural stress condition. *M. crystallinum* is used to compare the effect of salt stress and UV-B light. Plants, in hydroponical system, were subjected to 400 mM NaCl or/and to exposition to the range of 3 to 9 kJ m^{-2} d^{-1} UV-B_{BE} irradiation. The following day leaves and roots were used for analyses of polyamines and other metabolites changes.
Salt stress induced a general increase of polyamines either in leaves and roots, with also modification of composition. In particular the induction of cadaverine could have a role in resistance and adaptation. After UV-B irradiation the total polyamines showed a general decrease in root. In leaves increase of putrescine and spermidine seemed affected by the UV-B dose applied. Interesting is the absence of cadaverine after UV-B. The application of UV-B on plant under salt stress condition can block and modify the polyamine patter induced by NaCl. The data provide evidence that, at least on this plant, the UV-B stress caused polyamine responses divergent from that of salt stress. On the contrary, for many other cases the same metabolic responses under different stresses had been reported.
More then 25,000 ESTs sequences are available in *M. crystallinum* genome database. Only 20% of the most abundant transcripts in unstressed plants are also found in salt stressed. Several thousand transcripts in salt stressed state are under represented in EST collections from unstressed plants. Furthermore, are some of transcript related to different polyamine path presented by salt or UV-B stressed plants?

TRANSPORT OF GROWTH REGULATORS FROM ROOTS IN DRYING SOIL DURING PARTIAL ROOTZONE DRYING: THE MECHANICS OF A NEW DEFICIT IRRIGATION TECHNIQUE

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Decreased availability of water resources has increased pressure on crop managers to deliver improved crop water use efficiency into agriculture. To realise this aim, one strategy (termed "deficit irrigation") deliberately withholds water from plants. A recent form of deficit irrigation known as partial rootzone drying (PRD) aims to manipulate plant root-to-shoot signalling mechanisms to decrease crop water use. During PRD, water is distributed unevenly to the root system such that part is irrigated while the remainder is allowed to dry the soil. Theoretically, irrigated roots supply sufficient water to the shoots to prevent water deficits while the remainder sense drying soil and produce chemical signals that partially close the stomata and limit vegetative growth. However, the proportion of total sap flow (J) derived from drying roots (J_{dry}) may decrease as soil water potential (Ψ_{soil}) decreases, limiting transport of growth regulators to the shoots. The contribution of different parts of the root system to J and xylem ABA concentration ([X-ABA]) was investigated by grafting shoots onto two root systems of plants grown in two separate pots. The graft union resembled an inverted 'Y'. In PRD tomatoes, $[X\text{-}ABA]_{root}$ from the irrigated side underestimated $[X\text{-}ABA]_{leaf}$, while $[X\text{-}ABA]_{root}$ from the dry side overestimated $[X\text{-}ABA]_{leaf}$. The arithmetic mean of $[X\text{-}ABA]_{root}$ best explained variation in $[X\text{-}ABA]_{leaf}$, implying continued sap flow from the dry part of the root system at a Ψ_{soil} (-0.1 MPa) at which J_{dry} had ceased in a previous study. This was directly confirmed by sap flow measurements in grafted PRD sunflowers: J_{dry} began to decline when Ψ_{soil} = -0.18 MPa, and ceased when Ψ_{soil} = -0.55 MPa. Evaluating the relationship between J_{dry} and Ψ_{soil} may assist in maintaining export of ABA (and other growth regulators) from the drying part of the root system, to achieve desirable horticultural outcomes during PRD.

PLANT HORMONES IN CROPS UNDER STRESS: ENDOGENOUS RESPONSES AS THE BASIS TO DESIGN PALLIATIVE TREATMENTS

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The study of hormonal regulation of crop responses to abiotic stresses is crucial for modern agriculture. In this work, using citrus as a model crop, we studied the different patterns of hormone accumulation in response to water, salt and flooding stresses. Data revealed that ABA plays a central role as modulator of protective responses of citrus to abiotic stress whereas ethylene appears to act as an effector of the response. Jasmonates and polyamines also seem to be involved in the regulation of different events in woody plants under adverse situations. The understanding of the endogenous hormone regulation provided useful information for the development of palliative treatments with different hormone analogues on intact citrus plants under stress. In a first set of experiments, leaf abscission, chloride accumulation, ethylene production and net photosynthetic rate were the parameters used to characterize the performance of plants. Data indicate that exogenous applications of either ABA or a synthetic analog of this hormone were effective in delaying the deleterious effects of high salinity on citrus plants. The effect of agronomical treatments such as substrate amendment were also evaluated by examining endogenous hormonal changes. Current research in our laboratory follows a metabolomics approach to investigate the effects of stress on total metabolite profiles. High performance liquid chromatography coupled to mass spectrometry was used for metabolite profiling to assess physiological changes in response to environmental factors and/or endogenous signals with no previous information on the analyzed mass signals. In a parallel project, we are focusing in the annotation of mass signals and the search for markers linked to specific responses to environmental stresses.

IN VIVO TRAFFICKING AND LOCALIZATION OF p24 PROTEINS IN PLANT CELLS

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Normal plant growth and development are dependent on specialized tissue and subcellular-specific components of the plant membrane trafficking machinery. The plant secretory pathway, which is critical for biosynthetic and endocytic trafficking to the plasma membrane and vacuole, is comprised of the endoplasmic reticulum (ER), Golgi apparatus and intermediate organelles such as the prevacuolar compartment. Secretory membrane trafficking mechanisms have been shown to be involved in a variety of plant-specific processes, including abcisic acid and auxin signalling, plant development, tropic responses, and pathogen defense. We have focused in proteins of the p24 family, which constitute a family of putative cargo receptors which traffic in the early secretory pathway, although their precise function has not yet been established. Interestingly, these proteins have specific properties in plants, and may thus play plant specific roles. The p24 family can be divided into 4 sub-families (p23, p24, p25 and p26) by sequence homology. While mammals and yeast contain p24 proteins belonging to all 4 sub-families, all plant p24 proteins are of the p25 subfamily, characterized by the presence of a dilysine motif in the -3,-4 position and a pair of bulky hydrophobic residues in the -7,-8 position (with respect to the cytosolic C-terminus). We have previously shown that the cytosolic tail of Arabidopsis p24 proteins has the ability to interact with COPI (through the dilysine motif) and with COPII subunits (through the diaromatic motif). However, plant p24 proteins seem to have a higher affinity for COPI than for COPII. The aim of this work was to establish the localization and trafficking properties of a protein of the p24 family in plant cells and to investigate the contribution of the sorting motifs in its cytosolic tail to its *in vivo* trafficking and localization. Using a fusion protein between an Arabidopsis p24 protein and RFP (*At*p24-RFP), we have found that *At*p24 localizes exclusively to the ER, as its mammalian counterpart p25. Using mutant versions lacking either the diaromatic motif, the dilysine motif or both, we have found that the dilysine motif is necessary and sufficient for ER localization. In contrast, *At*p24 mutants lacking the dilysine motif are transported to the prevacuolar compartment and the vacuole, probably uncovering a default pathway for membrane proteins in the secretory pathway. Finally, our data suggest that while ER export of *At*p24 is COPII dependent, its ER localization requires COPI function, suggesting a highly efficient Golgi to ER recycling.

CROP WATER USE EFFICIENCY, CAN WE IMPROVE IT FURTHER BY MANIPULATING SOIL DRYING SIGNAL?

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Water use efficiency (WUE), if defined as the carbon assimilated over the water transpired (the physiological sense), is known as a conservative parameter (e.g. the difference between C_3, and C_4 plants) and largely a function of stomatal opening that determines the CO_2 concentration gradient from inside leaf to the outside atmosphere. Soil drying may lead to partially closed stomata (a better WUE) but also a reduced biomass accumulation as a trade-off. We found that irrigated plants tend to open their stomata fully and some narrowing of stomatal aperture from full may reduce water loss without much effect on photosynthesis. This is possible when part of the plant root system is irrigated while the rest part is left drying. A root 'drying' signal is then transported to the shoots where shoot physiology would be regulated. In our research over the last ten years, we have confirmed that root drying signal can be generated in the field for long term if a partial rootzone irrigation (PRI) is applied and plant water consumption can be improved as a result.

If we define the WUE as the yield over water irrigated (the agronomic sense), it should be a function of biomass accumulation, harvest index and the total amount of irrigation. Our field experiments presented a case that WUE may be enhanced through an improved harvest index. Harvest index has been shown as a variable factor in cases where whole plant senescence of rice and wheat is unfavourably delayed. Such delayed senescence can delay the remobilisation of pre-stored carbon reserves in the straw and results in lower harvest index. A controlled soil drying at grain filling time can enhance whole plant senescence and therefore improve the remobilisation of pre-stored carbon reserve. The gains from the improved harvest index may outweigh any possible biomass loss due to shortened photosynthetic period.

Related reviews:

Kang S, Zhang J. 2004. Controlled alternate partial rootzone irrigation: its physiological consequences and impact on water use efficiency. *Journal of Experimental Botany* **55**, 2437-2446.

Yang J, Zhang J. 2006. Grain filling of cereals under soil drying. *New Phytologist* **169**, 223-236. (Tansley Review).

SYNTHETIC PREPARATION METHYURE PROTECTS PLANTS UNDER SALT STRESS CONDITIONS

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Salinity is one of the hardest ecologic factors for plants represented a serious threat for agriculture in many countries including Ukraine. Global climate changes accrlerate its expansion especially on irrigated fields. Therefore increasing of plant salt tolerance becomes an actual practical problem which radical radical resolution consists in creation of salt tolerant transgenic forms of the main crop cultures. Besides there is a possibility of particular salt tolerance increasing with help of preparations capable to amlplificate adaptation processes in plant organisms. For this aim we have examinated some preparations and chose among them Methyure as a practically nontoxic and cheap synthetic compound tested before as a plant growth stimulator. As was found in our experiments its adaptogenic capacity is displayed at concentrations which are lower on some orders than ones provided a growth stimulation effect.

Protective mechanism of Mehyure has been investigated in comparison to synthetic preparation Ivine one on corn seedlings grown on Hoagland solution and exposed in NaCl presence. It was shown that seed soaking in 10^{-7}M water solutions diminished growth reduction and stress reaction indexes in NaCl-exposed sedlings. Protective effect of Methyure was stronger and realized mainly in roots whereas Ivine predominantly influenced shoots. Adaptation mechanism of these preparations consists in cell osmotic homeostasis supporting by soluble sugars and free aminoacids accumulstson accompanied by normalization ion homeostase due means of Na^+ transport influencing. Besides they can prevent stress peroxidation burst by activation of cell antioxidant system and by this way protect membrane structure and function.

Both preparations possess an antioxidant activity whereas Methyure has an additional antiradical property. However their efficacy in too low concentrations supposes that they don't act directly as antioxidants but their mechanisms are mediated on a genetic level.

Defence effect of these preparations during plant development has been studied in vegetation experiments on corn plants grown in vessels with soil contained 0.1M and 0,05M NaCl which are high concentrations for this culture. Salinity coused a death of plants at two month age but seed treating by Ivine couldn't prevent it. On the contrary Methyure provided surviving of the most plants during whole vegetation and permitted them to form cernels with seeds. It was found on one month plants that seed pretreating by Mrthyure protected their root system formation and nornalyzed ion balance in roots and leaves under salinity conditions.

It is known that plant organisms during their development have two periods of high sensitivity to negative factors what was confirmed by us under salinity conditions.We tried to support corn plants during their transition to generative period by their sprimkling by Methyure solution and this procedure showed perfect results.

Besides we carried out field experiments on slightly salinized soils and obtained results showed that under low salinity conditions. Not only Methyure but Ivine using too by two-time treating can provide a significant corn crop increasing.

Possibility of mass using of Methyure as antidepresant in agriculture on salinized soils and under other stress conditions has been discussed.

PRODUCTION OF PLANT HORMONES AND GROWTH REGULATORS BY ENDOPHYTIC MICROBES

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Endophytic microbes, which frequently emerge in plant tissue cultures, affect plant growth. For example, *Bacillus circulans* mediates somatic embryogenesis in *Pelargonium*, and many members of the genus *Methylobacterium* stimulate seed germination and promote plant growth and development *in vitro*. Production of phytohormones is a function typical for many plant-associated fungi and bacteria. The three types of growth-promoting plant hormones, auxins, cytokinins and gibberellins, are produced by mycorrhiza. Also fungal endophytes, such as *Colletotrichum*, can produce phytohormones. Production of plant hormones is also typical for many *Rhizobium* species and for the endophytic bacteria *Methylobacterium* spp., *Azospirillum* sp., *Acetobacter diazotrophicus*, and *Herbaspirillum seropedicae*. Endophytic microbes may act solely to increase the size of their habitat, but other, unknown mutualistic interactions may also exist. In animals, microbes are known to affect morphology, offer protection, and prepare a developing tissue for encounters with pathogens, but so far, very little is known of the interactions between the developing plant tissue and microbes.

GROWTH AND CYTOKININ CONTENT IN WHEAT PLANTS INOCULATED WITH CYTOKININ PRODUCING BACTERIA DURING RECOVERY AFTER DROUGHT

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In some experiments water shortage decreased cytokinins content in some plants (Hare et al, 1997, Kudoyarova et al., 2007) and this allowed us to hypothesise that plants may suffer from a deficit in cytokinins limiting their growth when they return to sufficient water supply. In order to test this prediction it was first necessary to follow the effect of drought on cytokinin content of wheat plants we worked with. We did find that water shortage decreased cytokinin content in the plants the effect being greater in roots than in shoots. The results suggested a decline in cytokinin synthesis by dried roots. Root/shoot cytokinin ratio was decreased by drought, which looked as if cytokinins were distributed in favour of shoots maintaining hormone content in the latter. The greatest drop in root cytokinins was observed in case of its transport form, which are ribosides (Mok and Mok, 2001) suggesting that the decline in root cytokinins in droughted plants may be due to export of hormones from roots to shoots. It is still possible that part of shoot cytokinins was synthesized in shoots of wheat plants themselves and there were indications that drought was likely to decrease production of cytokinins in shoots. Thus the content of phosphosylated cytokinins, in which form cytokinins are synthesised in plants (Mok and Mok, 2001), decreased more than that of other cytokinins in shoots of droughted plants.

The decline in cytokinin content in droughted plants was accompanied by and seemingly responsible for inhibition of shoot growth. The increase in water supply restored cytokinin content in wheat plants very quickly paralleled by an increase in relative leaf growth up to the level of continuously well watered plants. The only sign of cytokinin deficit was revealed one week after the increase in watering and manifested in lower content of phosphorylated cytokinins in shoots of previously droughted plants. It is possible that a longer and more severe drying of root zone may decrease ability of roots to supply shoot with cytokinins on re-watering, but in present experiments plants were able to increase cytokinin content up to the level of continuously watered plants. Nevertheless since droughted plants already had smaller leaves when water supply increased, they remained smaller one week later although during this week their relative growth rate was as fast as in continuously well watered plants. Thus after drought it was not sufficient to restore the level of cytokinins and growth rate. It was necessary to grow faster so that to catch up continuously well watered plants.

The results of inoculation of plants with cytokinin producing bacteria confirm that after normalization of water status plants needed more cytokinins. Inoculation of droughted plants prior to the increase in the level of watering led to an increase in the their cytokinin content obviously due to bacterially sourced hormones and in accordance with this they had higher growth rate and managed almost to reach the level of biomass accumulation of continuously well watered plants. It is of interest that cytokinins accumulated mostly in shoots of inoculated plants and not in roots, although they presumably arrived from the root zone. The results show that plants need additional cytokinins when they return from stressful to optimal environment and these cytokinins may be supplied by rhizosphere microorganisms.

The work was supported by RFBR (grant 05-04-50824)

PHOTOTROPHIC PURPLE BACTERIA AS MODEL SYSTEMS IN STUDIES OF CYTOKININS

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Free living phototrophic purple bacteria are no traditional object in cytokinin research in contrast to symbiotic and parasitic bacteria which exhibit phytohormon activity on plants.
Zeatin riboside and non-purine substance 4-OH- phenethyl alcohol with high cytokinin activity have been isolated from phototrophic purple bacteria *Rhodospirillum rubrum* several years ago by us. However, following studies of cytokinins in other purple bacteria showed absence of purine cytokinins even in that relating to the same α-phylogenetic group as *Rs. rubrum* and soil bacteria. It was supposed that the reason of this may be absence of the cytokinin biosynthesis genes or their expression. To check this point, we studied presence and functioning of ipt genes and transcription regulators of their expression in phototrophic purple bacteria.
Computer analysis of genomes on the level of amino acid sequences in phototrophic purple bacteria has shown the possible presence of plant type isopentenyltransferases carrying DMAPP to ADP/ATF. However, isopentenyltransferases of agrobacterial type performing transport of isoprenyl group to AMP has not been found. High resemblance of tRNA-isopentenyltransferase of *Agrobacterium tumefacience* and the same in purple bacteria mentioned above was observed. More highest likeness of tRNA-isopentenyltransferase of *Salmonella typhi* and purple bacteria *Rv. gelatinosus*, which have also similar structures of cell wall lipid A determining the toxicity of the pathogenic enterobacteria was revealed. The conditionally pathogenous *Rv. gelatinosus* might be a good object in monitoring the connection of bacterial toxicity and the structure of tRNA cytokinins.
High homology (up to 58%) of agrobacterial negative transriptional regulator, Ros, which controls ipt gene expression and the same in phototropic bacteria *Rps. palustris* have been revealed *in silico*. At present prokaryotic Zn-finger Ros proteins attract interest by their specific structure against eukaryotic one. In this connection the studies of structure and functioning of Ros-like protein in *Rps. palustris* will be very interesting and informative.
One more transcriptional regulator like of agrobacterial Vir A-Vir G ipt gene expression was investigated in phototrophic purple bacteria. It is known that VirA-VirG processing is initiated by acetosyringone (AS). This signaling molecule causes in laboratory agrobacteria cultures the excretion into culture medium the cytokinins in large quantity. Cytokinin-like substance 4-OH- phenethyl alcohol and hypoxanthine secretion, as the product of purine cytokinin degradation by cytokinin oxidase in *Rs. rubrum*, under AS treatment was observed. The possibility of cytokinin oxidase action has been shown by us early in transformed by ipt gene purple bacteria *Rps. palustris*. So, future studies of cytokinin oxidase structure and studies of ipt gene expression regulation by two-component transcriptional systems with using of wild type and transformed phototrophic purple bacteria are pioneering and resulting.

EFFECTS OF SERINE/TREONINE AND TYROSINE KINASE AND PHOSPHOTASE. INHIBITORS ON CORTICAL MICROTUBULE ORGANIZATION IN *ARABIDOPSIS* ROOT CELLS

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Microtubules (MTs), polymers of α- and β-tubulins, is one of the cytoskeletal components which involved in control of various processes of plant morphogenesis and development. As it was revealed for higher plants, α- and β-tubulins can be intensively regulated by post-translational modifications, including phosphorylation (Blume et al., 1997; Blume et al, *in press*). Plant tubulin phosphorylation is a reversible modification which could undergo as on serine/threonine (Ser/Thr) as well as on tyrosine (Tyr) residues; whereas till now its distinct role in plant cells was not clearly elucidated. It is known that level of tubulin phosphorylation in plant cells is determined by the balanced activity of protein kinases (PKs) and protein phosphatases (PPs). Therefore, to investigate the functional role of plant tubulin phosphorylation the effects of different types of inhibitors of Ser/Thr (H7, olomoucine (OM), staurosporine, W7) and Tyr (genistein, herbimycin A, tyrphostin AG 18) PKs, and inhibitors of Ser/Thr (okadaic acid (OA)) and Tyr (sodium orthovanadate (SO)) PPs on morphology of *Arabidopsis thaliana* primary roots and MTs organization have been studied. Effects of inhibitors were examined *in vivo* on *A. thaliana* line expressing GFP-MBD using confocal laser scanning microscopy (LSM 510 META, Carl Zeiss, Germany).

It was found that treatment of *A. thaliana* seedlings with effective concentrations of Ser/Thr PKs inhibitors reduced elongation of primary roots and caused alteration in root morphology. Also significant effects of all tested Ser/Thr PKs inhibitors on MTs organization in *Arabidopsis* root cells were observed. Treatments with W7, H7 and OM resulted in changes of cortical MTs orientation from transverse to longitudinal in epidermis and cortex cells of elongation and differentiation zones of roots. However, treatment with OA (an activation of protein phosphorylation process) resulted in MTs stabilization, changes of native MTs orientation or even in MTs disorganization in epidermis and cortex cells of elongation and differentiation zones. Also, OA affected root hair morphology; namely, root hairs swelling and branching as a result of abnormal MTs orientation were observed.

Inhibitors of Tyr PKs caused disorientation and disruption of MTs in epidermis and cortex cells of elongation and differentiation root zones, led to alteration of normal root hairs growth and development in comparison with untreated roots. Changes in MTs orientation from transverse to longitudinal in epidermis and cortex cells of elongation and differentiation zones, and intensive root hair development and growth were observed after treatment with SO (Tyr PPs inhibitor).

Thus, it was established that tested inhibitors of Ser/Thr as well as Tyr PKs and PPs cause significant effects on *A. thaliana* primary root morphology, root elongation and on MTs organization in different root cells. We suppose also that processes of tubulin phosphorylation/dephosphorylation can be involved in the dynamic and MTs organization in different types of living plant cells.

Blume YaB, Smertenko A, Ostapets NN, Viklickỳ V and Draber P. Post-translational modifications of plant tubulin. Cell Biol Int 1997; 21:918–920.
Blume Ya, Yemets A, Sulimenko V, Sulimenko T, Chan J, Lloyd C, Draber P. Evidence of tyrosine phosphorylation of plant tubulin. Cell Biol. Int. 2007 *In press*

SENSITIVITY TO PARAQUAT IN CYANOBACTERIA LACKING ANTIOXIDANT GENES

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The effects of herbicide paraquat, named also methyl viologen (MV), on the mutant of cyanobacterium *Synechococcus* sp. PCC 7942 lacking *sodB* gene and on the mutant of cyanobacterium *Synechocystis* sp. PCC 6803 lacking *katG* gene were examined. Cultures of the mutants and their wild type strains were grown in liquid BG11 medium enriched by 10 mM $NaHCO_3$ under 30 μmol photons m^{-2} s^{-1}, 28 ºC, and aeration by the air with 3% CO_2. Growth of the cultures was monitored by light scattering at 750 nm. In the linear stage of growth, cultures were centrifuged, resuspended in fresh medium to an A_{750} of 0.5, and maintained under the same conditions without (control) of with the presence of 0.5 μM MV. Damages of the photosynthetic apparatus were evaluated by measuring of CO_2-depended O_2 gas exchange in cell suspensions and by the delayed light emission (DLE) of chlorophyll *a*.
The in concentration of 0.5 μM was found to be a strong stressor for the *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 but had no significant negative effect on growth and photosynthesis in its wild type strain as well as in the wild type strain and the *katG*$^-$ mutant of *Synechocystis* sp. PCC 6803. Exposure to 0.5 μM MV inhibited growth, light-saturated O_2 evolution, and PS II activity (tested by DLE) in the *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 within 8 hr of stress treatment. In contrast, the wild type strain of *Synechococcus* sp. PCC 7942 remained nearly unaffected for 48 hr of 0.5 μM MV treatment. The oxidative damage to photosynthesis of the *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 was not accompanied by essential changes in chlorophyll content and carotene/chlorophyll ratio but was accompanied by greater catalase activity [1]. Earlier [2], it was demonstrated in this mutant that PS II activity, PS I cyclic activity, and the P700 reaction center are all targets of O_2^- formed at PS I and that the cytosolic superoxide dismutase (Fe-SOD) protects these targets from oxidative damage. The earliest of these targets to be damaged by MV in the absence of *sodB* gene is PS I cyclic electron transport. This observation is consistent with the vulnerability of Fe_4S_4 clusters of PS I to disruption by superoxide.
Present work suggests that lack of catalase activity in the *katG*$^-$ mutant of *Synechocystis* sp. PCC 6803 does not sensitize to MV because Fe-SOD is active. However, if the Fe-SOD is absent, as in the *Synechococcus* sp. PCC 7942 *sodB*$^-$ mutant, activation of catalase appears to be an adaptive response to MV stress that may allow survival, if not continued growth.
This work was supported by grant from the U.S. Civilian Research and Development Foundation; project # RB1-2351-PU-02.

References

Fomina I.R. *et al*. // Proceedings of the 6th International Symposium “New and Nontraditional Plants and Prospects of Their Utilization”, Moscow

Kozyrovskaya N.
Abstract not received

CHOLINE-CONTAINING GROWTH RETARDANTS INCREASE HEAT AND UV-B RESISTANCE OF PHOTOSYNTHETIC APPARATUS

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It is known that the use of growth retardants (GR) can enhance the plant resistance to stress-inducing factors such as drought, cold, salinity, ozone and УФ-В. However, it is little known about role of pre-treatment by GR in stress resistance of the photosynthetic apparatus (PA). Our objective was to study the influence of GR – choline-containing compounds, such as 2-chloroethyl-trimethylammonium chloride (CCC) and its analogue 2-ethyl-trimethylammonium chloride (CC) on stress resistance of the photosystem II (PS II). Photochemical activity of PS II was assessed by measurements of chlorophyll *a* variable and delayed fluorescence using phosphoroscope. The activities of antioxidant enzymes (catalase, ascorbate peroxidase, total peroxidase and glutathion reductase) and contents of pigments (carotenoids and flavoinoids) were determined spectrophotometrically by corresponding methods. The levels of abscisic acid (ABA) and cytokinins were assessed by enzyme–linked immunosorbent assay. We have showed that the application of CCC and CC both to bean seeds and seedlings diminished UV-B and heat induced inhibition of PS II activity (1). Membrane thylakoids isolated from the primary leaves of seedlings treated with choline-containing compounds were also more resistant to UV-B. The higher resistance of the PS II in beans pre-treated with choline-containing compounds assessed by the ratio (F_v/F_m) and maximal intensity delayed fluorescence of Chl *a* correlated with increased activities of antioxidant enzymes and higher amount of low molecular antioxidants such as carotenoids and flavonoids detected by us in primary bean leaves. Total peroxidase activity in thylakoid membranes isolated from leaves of pre-treated seedlings increased twice. Pretreatment with choline compounds increased contents of cytokinins (2) and ABA in leaves as well. We suggest that enhanced stress resistance of the PS II in plants treated by GR might be due to increased activities of the antioxidant enzymes as well as an increase in the content of low-molecular antioxidants (carotenoids, flavonoids) and hormones (ABA, cytokinins). The one of the reasons of high antioxidant activity in leaves and membrane thylakoids can be weak stress that develops as a result of choline pretreatment. Such weak stress was indicated in cells of green alga *Chlamydomonas* (3) treated with cholines.

1. Kreslavski V.D, Balakhnina T.I, Khristin M.S, Bukhov N.G. Photoynthetica. 2001. 39: 363-368.

2. Kreslavski V, Kobzar' E, Ivanova E, Kuznetsov E. Plant Growth. Reg. 2005. 47: 9-15.

3. Ladygin V.G., Shirshikova G.N., Semenova G.A., Kreslavski V.D. Bioph. 2001. 46:256-264.

CYTOKININ SECONDARY HORMONE AND 14-3-3 PROTEINS ACTIVATES THE CA^{2+} ATP-ASE PUMP AND THE CYTOSOLIC CA^{2+} SWITCH ON THE ACTIVITY OF AMMONIA ASSIMILATION NADP -GLUTAMATE DEHYDROGENASE OF SPHEROSOME IN THE FILLING WHEAT GRAINS

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Our works is devoted to investigation of signal transduction of cytokinin. Earlier it was shown by us that cytokinin induces the formation of strong NADP-dependent GDh electrophoretic forms in whole wheat grain. In the same time the cytokinin inducing effect absent when cytokinine acts on unembryonated wheat seeds. In this reason it was assumed by us that cytokinine induces in the seeds embryos the formation of cytokinin`s secondary hormone (CSH). The CSH was purified from germinating wheat seeds by hydrophobic chromatography on column with octylsepharose 4B and by reverse phase chromatography on column type RP-18. It was shown that CSH is very close by its properties to fusicoccin.

So, the purified CSH strong inhibited the binding of tritium-labeled fusicoccin with fusicoccin receptors from roots of Zea maize seedlings**.** It was shown that CSH and 14-3-3 proteins activates Ca^{2+}- dependent ATP-ase of plasmatic membrane from aleuron layer of wheat seeds and increases the level of cytosolic Ca^{2+}. It was established that cytosolic Ca^{2+} activates NADP-GDH of spherosome in aleuron layer of wheat seeds. Using Ca^{2+} ionophore A_{23187} it was shown that artificial increasing of the level of cytosolic Ca^{2+} induces the formation of NADP-GDh of spherosome without effect of CSH whereas the artificial decreasing of the level of cytosolic Ca^{2+} canceled the effect of CSH. Thus level of cytosolic Ca^{2+} plays determining role in the activation of NADP-GDh of spherosome.

Thus we discover that the intracellular target for action of cytosolic Ca^{2+} ions is subcellular organelle - spherosome.

It was established that NADP-GDh of spherosome from filling wheat grains has the very high affinity to ammonia. Km to ammonia of NADP-GDh is equal 1,3 mkM. Dephosphorylation of this NADP-GDh let to decreasing the affinity of this enzyme to ammonia. Its necessary to know that the NADP-GDh activity shows only spherosomes as whole structure, but we don't be able to find this activity in soluble state. Thus it was shown that CSH and 14-3-3 proteins activates the Ca^{2+} ATP-ase pump and the cytosolic Ca^{2+} switch on the activity of ammonia assimilation NADP - GDh of spherosome in the filling wheat grains.

Our experimental data allow us to suggest the next scheme of signal transduction of cytokinin. Cytokinin induces the formation of CSH, then CSH activates the formation of 14-3-3 proteins. After translocation CSH and 14-3-3 proteins are bonded with receptors of Ca^{2+} - ATP-ase of plasmatic membrane. Then this gives the increasing of level of cytosolic Ca^{2+}. Ca^{2+} is bonded with GDh of spherosome and activates the NADP-GDh and its phosphorylation.

UKRANIAN PLANT GROWTH REGULATORS: FROM IDEA TO REALITY

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During 20 years since foundation of Bioorganic Chemistry & Petrochemistry Institute of NAS of Ukraine, we have developed the whole series of high efficiency plant growth regulators of new generation.

On the basis of the fundamental research results the authors offered the hypothesis concerning universal and narrow specific action of exogenous regulators on the plant growth processes. Regulators act through changing the synthesis of phytohormones which are accounted for the recognition and regulation of gene activity. These processes promote the development and growth of plants.

Hypothesis also explains the possible mechanism of the universal action of exogenous and endogenous plant growth regulators.

New plant growth regulators activate the basic of vital functional of plants, quality of products is improved, plant stability to the diseases and damages by insects are promoted content, content of nitrates, ions of heavy metals and radionuclide in products are lowered, mutagenic action of herbicides and other anthropogenic factors is descended in two times.

Developed regulators are ecologically safe. They positively influence on development of soil micro flora, growth of the root system, leaf surface and photosynthesis are strengthened, and stability to the stress factors (frost, drought, soil salinity) is promoted.

New plant growth regulators on the efficiency correspond with the best world standards, and on the technological index and the cost they are surpassed considerably. Cost of growth regulators application per 1 hectare almost to 3-7 $ USA.

The ISTC "Agrobiotech" was created in 2000 on the basis of Bioorganic Chemistry and Petrochemistry Institute for production and creation new plant growth regulators and now produce 30 items regulators for agrarian complex.

THIAZOLIUM ION: RING OPENING AND ACTIVITY IN MODEL SYSTEMS

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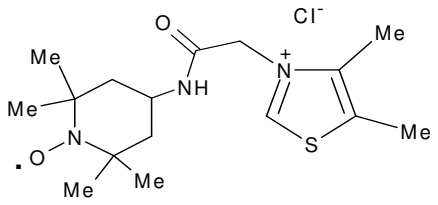
Much of the attention directed to mechanistic aspects of biological transport of thiamine has focused on mechanisms of reversible hydrolysis of thiazolium ion. We have investigated the kinetics of thiazolium ring opening of thiamine and its structural analogues in the presence of 5,5′-dithiobis(2-nitrobenzoic acid). It was found that values of the reaction rate constants catalyzed by general base correlate with inductive effect of substituents at position 3 of thiazolium cycle of 3-R-4-methyl-5-(2-hydroxyethyl)thiazolium ion, including 4-amino-2-methylpyrimidinyl-5-methyl substituent of thiamine.

Because of biochemical importance of thiazolium ion hydrolysis the properties of N-[(1-oxyl-2,2,6,6-tetramethylpiperidinyl-4)aminocarbonylmethyl] substituted thiazolium salts I, II in aqueous micellar solutions (physiological pH range) in the presence of sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) have been studied by EPR spectroscopy. Binding of paramagnetic thiazolium salts to the SDS and CTAB micelles resulting by increase of apparent rotational correlation time (τ_c) depends on the nature of the micelle, hydrophobisity of substituent in position 5 of thiazolium cycle, temperature and pH of solution. Dependences of free radical $\ln\tau_c$ on 1/T at pH 6 and pH 7,6 were analysed. The increase of τ_c at interaction of ion II with CTAB micelles at pH 7,6 in comparison with pH 6 is caused by thiazolium ring opening and formation of thiol form of nitroxide. Substantial increase of τ_c and relative parameter of hydrophobisity in the presence of 5,5′-dithiobis(2-nitrobenzoic acid) may be due to accumulation in CTAB micelle of mixed disulfide.



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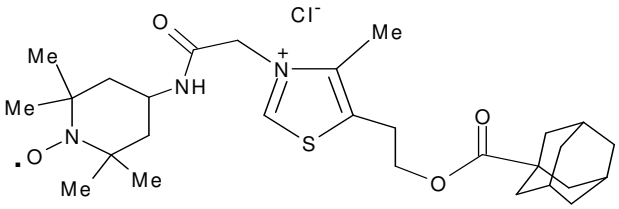

II

We have examined also the binding of 3-[(1-oxyl-2,2,6,6-tetramethylpiperidinyl-4)aminocarbonylmethyl]-4-methyl-5-[2-(1-adamantoyloxy)ethyl] thiazolium chloride to the thylakoids of pea chloroplasts. Binding of spin probe to the membrane structures is revealed by significant increase of τ_c in a range of 20-60^{o}C.

It was established that cyclic photophosphorylation with phenazine methosulfate in isolated pea chloroplasts is inhibited by 3-benzyl-4-methyl-5-(2-acyloxyethyl) thiazolium salts containing at position 5 the acyl fragment of norbornane-2-carboxylic, adamantane-1-carboxylic, adamantane-2-carboxylic, adamantyl-2-acetic, 5-methyladamantyl-1-acetic and diphenylacetic acids. The degree of inhibition of ATP formation was increasing at combined action of the thiazolium salts and cationic detergent - cetyltrimethylammonium bromide - in concentrations which did not provide considerable effect of these compounds separately. Dependence of chloroplasts activity inhibition on the nature of substituents at positions 3 and 5 thiazolium ion was analysed. It was concluded that thiazolium cation localization on the membrane of thylakoids followed by thiazolium ring opening and interaction with ATP synthase causes inhibition of photophosphorylation.

For 125 years
N.G. Kholodny devoted:

N.G. KHOLODNY'S IDEAS AS A TRIGGER OF THE PHYTOHORMONOLOGY DEVELOPMENT

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«About a quarter of a century ago… there were laid foundations of a new science of plant endocrinology, or, in other words, the science of plant hormones (phytohormones). Within a short time span this young science achieved much and succeeded in studying the chemical nature and physiological role of those substances. Some plant hormones have been isolated and produced as pure chemicals. That enabled synthesizing chemical compounds similar in their activity to natural phytohormones; however, these new substances have never been found in plants. Those were so-called synthetic plant growth substances."

Those words were written in 1949 by Academician M.G. Kholodny, a great naturalist and thinker of the 20th century, one of the founders of phytohormone science and creators of the hormonal theory of tropisms. On June 22, 2007, the scientific community of Ukraine celebrated the 125th Anniversary of this outstanding naturalist, known for his profound and pioneering studies in the fields of plant physiology, microbiology, ecology, philosophy, and general biology (natural sciences), who not only established the modern plant endocrinology but also predicted the main trends of development of this science.

In view of the aforesaid, I would like to remind you that already in 1933 Kholodny formulated the concept of «hormone»: "A plant hormone should be regarded as a substance that is produced in a plant organ, is able to get into growing tissues, and control, that is to increase or decrease, the rate of cell growth, acting in trace quantities". Phytohormones are substances-inductors, organizers of growth processes and morphogenesis, which "…with some combination of internal and external conditions, despite their minute content in the organism, acquire an ability to change the rate and direction of physiological processes occurring there".

It should be emphasized that Kholodny established fundamentals of the science of phytohormones at the time when only one plant hormone, auxin, was known. But even in that time he already spoke about polyvalent functions of the plant hormone, came up with an idea of a great variety of hormones in the plant world. He wrote: «We should bear in mind that, in addition to auxin, the plant organism contains some other substances of hormonal types». In view that he foresaw that «…various morphogenetic processes, and plant development in general, must be related to the action of phytohormones appropriately distributed in the plant organism».

As a result of M.G. Kholodny research, there was established a physiological polyfunctionality of auxin (as he wrote, «auxin polyvalency»), its ability to cause various effects depending on its concentrations and nature of a substrate on which that substance acts.

He experimentally demonstrated that a growth substance «plays an important role in the mechanism of geotropic responses in general. Plant organs whose upper tips do not perform any specific physiological functions, "irritants" are not exceptions in this respect».

In 1918 M.G. Kholodny expressed new ideas on the localization of synthesis of phytohormones and their transport: «The root tip is an internal-secretion organ that exudates into the growth zone some substances of a hormonal type». Later that suggestion was proved by classical experiments on sections of roots, hypocotyls and coleoptiles of corn, oats, and lupine. Experimental studies carried out by M.G. in the 1920s-1930s to investigate the effects of hormones on the root growth enabled him to show for the first time that phytohormones can not only stimulate but also inhibit plant growth: "a substance diffused from the corn coleoptile contributes to the coleoptile growth and at the same time quite evidently inhibits the root growth".

Through his experiments M.G. Kholodny discovered one more significant scientific fact concerning the speculative «irritant» itself – the substance that was produced by corn coleoptile apical cells and that was classified as a plant hormone. That was the phenomenon of non-specificity of hormonal action: "…substances that are produced by cells in upper tips of *Zea mays* coleoptiles are by no means specific".

While continuing those experiments, M.G. paid attention to the fact that "a disruption of the hormonal balance may cause development of tumors (swellings) in plants" followed by development of lateral, auxiliary roots. Thus, it was the first demonstration of the morphogenetic effect of plant hormones.

Analyzing the results of his experiments that showed some morphogenesis disruptions in roots resulting from auxin effects, M.G. expressed hope that the introduction of "phytohormones into the plant organism at the specific stages of its development may in the future be a very effective method to increase the production of plant mass and to control development of agricultural plants". Observing a high physiological and biochemical activity of phytohormones, M.G. concluded that "that peculiarity … makes them the most suitable means for changing the course of various life processes".

We are all witnesses that these visionary views of M.G. have not only been proven but become commonly accepted, widespread ideas. It should also be added here that in 1948 M.G. put forward a new concept of parahormones, the substances that are not typical of plants but are obtained synthetically and characterized by a high physiological activity. And only much later it has became clear that it was an outline for a new direction in studies of plant growth and development: using synthetic analogues of phytohormones for scientific and practical purposes. Thereby, the foundation was established for the advent of a unified concept of regulators of plant growth and development.

One of the most significant problems of phytohormonology is the role of phytohormones in plant transition from the vegetative stage to the generative one. M.G. was not directly involved in such research; however, already in 1938 he published an article entitled "Does the flowering hormone exist?" in which he assumed that "not some specific substance but the whole complex, compounds of known metabolites, possibly including hormones, cause the setting and development of buds". Fifty years later, in 1988, this idea proposed by M.G. was formulated by the Belgian scientist Bernye in his multifactor theory of flowering control, according to which the floral stimulus is a combination of assimilates and complex of known hormones.

It is very much to the point to conclude this brief description of Kholodny's ideas and previsions, which have become not only the beginning of modern phytohormonology (phytoendocrinology) but also have determined for many years the directions of further development of this science, by his words from the conclusions of his famous worldwide (but presently rare) book "Phytohormones. Essays on Physiology of Hormonal Phenomena in a Plant Organism".

The significance of «"infinitesimals", both in the scope of problems concerning the structure of «living matter» and regarding biochemical and physical processes occurring in plant and animal organisms, increases in modern biology every year. In the last field this basic trend in modern natural science is expressed in an intense interest in various oligo-dynamic phenomena, that is, in the processes involving nearly imperceptible minute quantities of substance and energy. It becomes more and more clear that just those phenomena form a typical feature of the organized nature, and that without a complete and precise knowledge of them, the final aim of biological science can not be achieved. This aim is "getting control" of living nature in theory and in practice, that is, an ability to predict events that will occur in it and control them in conformity with our interests and knowledge.

I would like to stress the fact that all the above-mentioned citations were written before the year of 1939; that is, long before the mankind could use nuclear power, before the advent of molecular biology and modern nanophysics, before clear understanding of the structure of

living matter at all levels of its organization. It is a direct evidence of the scientist's genius, the scope of his scientific interests, understanding of the basic tendencies in development of science, and richness of his erudition.

1. Cholodny N. Ueber die hormonale Wirkung der Organspitze bet der geotropisschen Krümmung / Ber. D. deut. Bot. Ges. – 1924. – 42. – P. 356-362.
2. Cholodny N. Wuchshormone und Tropismen bei den Pflanzen / Biol. Zentralbl. – 1927. – 47. – P. 604-626.
3. Cholodny N. Zur Physiologie des pflanzlichen Wuchshormons / Planta. – 1931. - 14. – P. 207-216.
4. Cholodny N. Zum Problem der Bildung und physiologischen Wirkung des Wuchshormons bei den Wurzein / Ber. D.deut. bot. Ges. – 1933. – 51. – P. 85-98.
5. Cholodny N. Ueber das Keimungshormon von Gramineen / Planta. – 1935. – 23. – P. 289-312.
6. Kholodniy N. Phitohormones on physiology of hormonal phenomena in a plant organism. – 1939. – Kyiv. Academy of Science of Ukraine. - 265 p. (in Russian)
7. Musatenko L.I. The hormones of algae // International Journal of Algae. – 5. – 7 (1). – P. 5-22.
8. Problems of Phytohormonology / Ed. K.M. Sytnik. – Kyiv: Phytosociocenter, 2007. – 420 p. (in Ukrainian)
9. Sytnik K.M., Musatenko L.I., Vasyuk V.A., Vedenicheva N.P. et al. Hormonal complex of plants and fungi. – Kyiv, 2003. – 186 p. (in Ukrainian)
10.

ABSTRACTS
POSTER PRESENTATIONS

SESSION 1:
Mechanisms of cytokinin signaling and action

EXPRESSION OF GENES PARTICIPATING IN THE CONTROL OF PLANT CELL DIVISION IN TUMOR-PRODUCING RADISH (*RAPHANUS SATIVUS* VAR. RADICULA PERS.) INBRED LINES

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Tumors in higher plants are suitable model for studying mechanisms of systemic control plant cell division and differentiation. Probably, tumor formation is under control of numerous genes. On spontaneous and pathogen-induced tumors in different plant species it was shown that the key role in tumor formation plays alteration of cytokinins/auxins ratio. In the other hand, tumor formation is accompanied by change of expression levels and patterns next of genes that participate in cell cycle control, homeobox-containing genes controlling meristem cell specifity and genes of primary response to auxins and cytokinins.

Spontaneous tumors on inbred lines from radish (*Raphanus sativus* var. Radicula Pers.) genetic collection are used for study the mechanisms of tumor growth in higher plants. Two types of tumors were described for radish lines: undifferentiated tumors which form on the crop-roots of several lines during flowering and tumors on ovaries which undergo redifferentiation and form ectopic shoot meristems. Besides that, cultivation of young aseptic plants from many lines of radish on the mediums with cytokinins leads to formation of tumors in the lower part of hypocotyl. These tumors are close resemble to tumors in the crop-roots in their anatomy and are capable to hormone-independent growth for a long time. It was shown that lines which are tumor-producing *in vivo* have increased level of free and bound forms of cytokinins and decreased level of IAA. Explants of tumorous lines demonstrate high sensitivity to exogenous auxins and cytokinins *in vitro*.

We have used RT-PCR to analyze expression of some cell cycle genes (*CycD3*), meristem-specific genes (*STM, KNAT1, WUS)* and primary response genes to auxins (*IAA1*) and cytokinins (*ARR5*) during tumor formation in radish lines. PCR-products which were obtained during PCR of radish the DNA and cDNA with specific primers to *CycD3, STM, KNAT1, WUS IAA1* and *ARR5* genes demonstrated high level of homology with the corresponding genes of *Arabidopsis thaliana*. Expression of named genes was studied on different tissues of lines producing tumors in the crop-roots, lines forming ovary tumors and non-tumorous lines in several stages of development; besides that we have analysed their expression during formation cytokinins-induced tumors on aseptic plants. The expression of *CycD3* and *KNAT1* genes was increased during the development of spontaneous tumors on the crop-roots and cytokinins-induced tumors. Formation of ovary tumors was accompanied by increased expression levels of *CycD3, STM* and *WUS* genes. The treatment of radish plants by cytokinins induces the incease of *ARR5* gene expression level. We have shown that the expression of *ARR5* is induced early and stronger in tumorous lines. In line which has maximal rate of tumor formation, we observed high level of *ARR5* expression without any cytokinins treatment. On the contrary, increased level of *IAA1* expression we observed in non-tumorous lines. So that, *CycD3, STM, KNAT1, WUS* and *ARR5* genes may participate in the control of tumor development on radish lines.

The work was supported by RFBR 05-04-48583, CRDF ST-012-0 и CRDF BP2M12 grants.

PARTICIPATION OF PIP_2-PHOSPHOLIPASE D IN CYTOKININ SIGNALING

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Cytokinins are class of plant hormones that play central role in many aspects of plant growth and development including apical dominance, formation and activity of shoot meristems, flower and fruit development, leaf senescence, source-sink relations, photosynthesis, seed germination, control of cell division, pigment production and plant defense responses. They also appear to mediate a number of light-regulated processes, such as deetiolation and chloroplast differentiation. For cytokinin signaling, most clear evidence was obtained on the involvement of Phospholipase D (PLD). This evidence is based on a dose-dependent inhibition of cytokinin-induced pigment accumulation in *Amaranthus caudatus* L. by low concentrations of primary alcohols, known specific inhibitors of phosphaditic acid formation by PLD. Secondary alcohols, which do not interfere with PLD action, lack such inhibitory effect. Also, primary alcohols partially prevented cytokinin-responsive *ARR5* gene promoter activity and reduced an accumulation of *ARR5* gene transcripts in *Arabidopsis thaliana*.

Phospholipase D (PLD) constitutes a major plant phospholipase family in plants involved in many cellular processes such as signal transduction, membrane remodeling, and lipid degradation. Stimulation of PLD also has been shown in plants in response to variety stress and hormone treatments.

The effect of cytokinin (BA) on the phosphatidylbutanol (PtdBut) accumulation was tested in tissues of *Amaranthus caudatus*. Detached shoots of *Amaranthus* were placed in flasks containing [^{33}P]orthophosphate and incubated for 14 h at 25 ˚C. BA was added at different time-points, and lipids were extracted and analyzed. PLD activity was measured according to phosphatidylbutanol accumulation upon 1-butanol addition. Cytokinin application during 5-30 min caused increase in phosphotidylbutanol accumulation.

In order to reveal the type of PLD activated upon cytokinin action, we used different modifiers of signaling cascades: EGTA that binds calcium, verapamil that inhibits plasma membrane calcium channels and neomycin that binds PIP_2 and inhibits PLD activity. The modifiers were applied together with 5 μM BA. As the result, EGTA, verapamil and neomycin blocked cytokinin-induced PtdBut formation in the following way: neomycin > verapamil > EGTA. Therefore, among the inhibitors tested, the neomycin was the most potent in reducing PtdBut levels. These results indicate that PLD activated by cytokinin can be PIP_2-dependent.

This study was supported by the grants: INTAS N602/2001 and NAS of Ukraine № 2.1.10.32-05.

FUNCTIONAL HETEROLOGOUS EXPRESSION OF *ARABIDOPSIS THALIANA* CYTOKININ OXIDASE/DEHYDROGENASE FAMILY

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Cytokinins are plant hormones able to promote cell division, therefore contributing to the regulation of a variety of developmental processes including apical dominance, flower and fruit development, leaf senescence, and seed germination.
The degradation metabolism of cytokinins is an important process that controls the levels of this hormone active forms and their distribution in plant tissues. The enzyme catalyzing the cleavage of N^6-side chain of free cytokinin bases and their ribosides is a flavoprotein classified as a dehydrogenase, but able to work also in an oxidase mode (CKX, EC 1.5.99.12). In *Arabidopsis thaliana*, seven distinct CKX-encoding genes were identified. Amino acid sequence comparison revealed that individual CKX proteins from *Arabidopsis* share conserved regions of high homology (e.g. FAD-binding domain), but their sequences outside of these domains display strong divergence. Therefore these isoenzymes differ in their catalytic properties, their subcellular localization and their expression domains.
Functional expression of several recombinant AtCKX proteins has been obtained successfully in the *Pichia pastoris* system, with protein secretion to the medium. In the case of AtCKX1 and AtCKX3, N-terminal sequence-specific vacuolar sorting signal (ssVSS) was identified that had to be deleted to achieve secretion of active proteins from the yeast cells.
In order to facilitate purification, recombinant proteins were fused with His-tag domain. The fusion on the C-terminal end of the proteins, however, proved to be useless in affinity purification, suggesting that polyhistidines are buried inside the protein structure and cannot bind to the metal-chelating resin. Proteins with N-terminal His-tag domain are retained on Ni-NTA columns but the binding strength differs significantly for each protein.
In neutral conditions the dehydrogenase activity of AtCKX enzymes is the highest with N^6-(2-isopentenyl) adenine (iP), whereas in slightly acidic pH most enzymes prefer iP9-glucoside. Oxidase activity is the highest with iP9-glucoside in neutral conditions. Aromatic cytokinins and their ribosides are relatively good substrates only for AtCKX1 and AtCKX3 in slightly acidic conditions with 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q_0) as the electron acceptor.

DIMETHYLSULFOXIDE SELECTIVELY STRENGTHENS THE CYTOKININ ACTION IN ARABIDOPSIS

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Dimethylsulfoxide (DMSO) is one of the widespread solvents used for pharmacological screening, in biology in particular. Also DMSO can affect biological membranes and can be employed for membrane permeabilization. We have used DMSO previously to study the influence of different growth substances/inhibitors on the expression of *ARR5::GUS* construct in transgenic Arabidopsis (Romanov et al., FEBS Letters, 2002). The construct represents the fusion of the promoter from the cytokinin primary response gene *ARR5* with the reporter gene *GUS* (Brandstatter & Kieber, Plant Cell, 1998). We remarked that in control series some concentrations of plain DMSO influenced somehow cytokinin effects. For example, DMSO at concentration 2-7% enhanced cytokinin action by 2-3-fold. Therefore we have checked the influence of different DMSO concentrations on the induction of *ARR5* promoter by cytokinins, using different transgenic clones of Arabidopsis. For experiments, we have used 3-4-day-old seedlings of double Arabidopsis mutants expressing only one of three cytokinin receptors (Riefler et al., Plant Cell, 2006): *ahk2/ahk3*, *ahk2/ahk4* and *ahk3/ahk4* expressing sole receptors CRE1/AHK4, AHK3 or AHK2, respectively. These mutants were additionally transformed with *ARR5::GUS* construct (Riefler et al., unpublished). Quantitative GUS assays have shown that double mutants differed in their response to DMSO. The mutant *ahk2/ahk4* (expressing AHK3) seemed to be most responsive (5-fold increase at DMSO concentration 4.2%), the mutant *ahk3/ahk4* (expressing AHK2) less responsive (2-fold increase at the same DMSO concentration) and the mutant *ahk2/ahk3* (expressing CRE1/AHK4) almost non-responsive. Direct binding assays have shown no any positive influence of DMSO on the affinity of receptors to cytokinins.

In order to study this phenomenon in more detail, we employed the histochemical staining of Arabidopsis on GUS activity. Seedlings were incubated on DMSO solution (4%) or DMSO together with cytokinin (BA, 5 μM) for 5 h. Seedlings grown on water or BA solutions served as controls. Our preliminary data showed difference in the pattern of GUS activity between mutants. For example, in all experimental and control series with *ahk2/ahk3* mutant only the extensive coloring of root (especially root tip) was obvious, but not of hypocotyls or cotyledons. The clone *ahk2/ahk4* showed, on the contrary, marked coloring in hypocotyl and cotyledons. This coloring became more intensive after DMSO treatment.

These results give evidence that the positive influence of DMSO on cytokinin action is rather selective depending on the type of expressed cytokinin receptor. The different responsiveness of mutants can be, at least partly, explained by the different subcellular localization of the receptors.

Supported by grant RBFR (Russia) N 07-04-00331

TWO MAIZE CYTOKININ RECEPTORS, ZmHK1 AND ZmHK2, HAVE DIFFERENT LIGAND-BINDING PROPERTIES

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Cytokinins are "classical" plant hormones that regulate various processes of plant growth and development. The molecular mechanism of cytokinin signaling is now under extensive studies. At present, it is commonly assumed that cytokinin signal is transduced via two-component system, with hybrid sensor histidine kinases as receptors. Three cytokinin receptors were characterized from maize, ZmHK1, 2 and 3a (Yonekura-Sakakibara et al., Plant Physiol., 2004). The ZmHKs were expressed in the Δ*rcsC* and *cps::LacZ* mutant background of *E. coli*. ZmHK1 and ZmHK2 were able to complement the function of RcsC in a cytokinin-dependent manner. Hence these receptors obviously retained their functional state upon expression in bacteria.

Using transformed bacterial cells, we investigated cytokinin-binding properties of ZmHKs by a direct radioligand method (Romanov et al., 2005). Highly labeled ^{3}H-*trans*-zeatin served as a ligand. Both receptors bound *trans*-zeatin with high affinity: dissociation constants (Kd) for ZmHK1 and ZmHK2 corresponded to approx. 80 and 1 nM, respectively. Receptors had different preference for some cytokinins. Among numerous substances tested ZmHK2 displayed high affinity to only *trans*-zeatin. By contrast, ZmHK1 tightly bound also isopentenyladenine (iP, Kd 5 nM) and benzyladenine (BA, Kd 12 nM). Functional test on the *cps::LacZ* construct activation by different cytokinins showed reasonable correlation with binding data.

The influence of media conditions on cytokinin binding to receptors was also investigated. We found that mono- and divalent cations (K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, Mn^{2+}) at physiological concentrations had no marked effect. The receptors differed in pH-dependence of ligand binding. The binding activity of ZmHK2 was almost not affected by pH change (from 5 to 9). On the contrary, ZmHK1 was shown to be strong pH-dependent. We supposed that difference in pH-dependence of hormone-binding properties might be due to different subcellular localization of receptors. Our first results revealed some difference in cytokinin binding sites between plasma- and inner (ER) membranes. Such difference might indicate that ZmHK1 is mainly located on inner membranes, whereas ZmHK2 on plasma membrane.

Thus, maize cytokinin receptors ZmHK1 and ZmHK2 are quite different in their ligand-binding properties. This makes clear parallel between maize cytokinin receptors and corresponding receptors from Arabidopsis, AHK3 and CRE1/AHK4. The revealed differences in ligand-binding properties might be connected with different pattern of *in planta* expression and/or different intracellular localization of cytokinin receptors.

Supported by grant RBFR (Russia) NN 07-04-91211-ЯФ

THE PARTICIPATION OF CYTOKININS IN THE GENERAL ADAPTATION SYNDROME IN WINTER WHEAT

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Cytokinins are a class of plant growth regulators that plays a key role in different processes of development such as control of apical dominance in the shoot, shoot morphogenesis in cell and tissue culture, root growth, chloroplast development, leaf senescence. It is known that phytohormones take a part in the regulation of plant stress too. This is supposedly triggered by hormonal changes such as increased levels of ABA and decreased concentrations of cytokinins. Although a role for ABA in mediating many physiological responses to environmental stress is now well-established, evidence has been presented for the existence of five signal transduction pathways that regulate drought- and cold-inducible genes, only two are dependent on ABA action. Furthermore, recent studies had been shown that some stress-related genes might express under high cytokinin concentrations. Therefore the aim of our research was the investigation of the possible participation of cytokinins in realization of stress responses.

We studied the effects of high temperature (38-40°C) on the balance of two cytokinin forms (zeatin and it riboside) in leaves, transpiration rate and root meristematic activity of eight-day-old plants of four winter wheat cultivars - Khar'kovskaya 81, Khar'kovskaya 96, Donetskaya 46 and Polukarlik 3 – after 1-, 2-, 5-, 10-, 15-, 30-, 45-, 60- and 90-minutes exposition.

Under the optimum temperature (22-24°C) the content of zeatin and zeatin riboside was not changed dramatically in the leaves of both cultivar, but we observed some oscillation of them. Probably it is caused by the day periodic activity of plants, because our experiment started at 12 a.m. High temperature had the positive effect on the zeatin concentration in leaves tissues of all cultivars, although the time reaction was markedly different. Then zeatin content was decreased rapidly. At the same time zeatin riboside dynamics had another character – it was decreased after the start of stress and increased in the second half of hour. Perhaps the pool of zeatin riboside is the reserve for zeatin in leaves but this reserve is not enough for the maintenance of concentration of physiological active forma of cytokinins.

Transpiration rate had been changed two times during the experiment. At the beginning of experiment transpiration rate had been decreased and the first maximum of evaporation coincided with the peak of cytokinin content in the leaves, but the second one was differed from it one.

Meristematic activity had been decreased under high temperature conditions in roots of all cultivars. But the long-stemmed cultivars had prepotent and faster reduction of meristematic index than semi-dwarf one. At the same time we observed the increase of relative duration of prophase and telophase when the anaphase and metaphase one were reduced substantially.

So it is possible that at the first minutes of high temperature stress the synthesis and transport of active forms of cytokinins were not inhibited and they supported cell division in root meristem. However the cells were remarkable by their competence towards cytokinins and ABA that might result in the stomatal guard cells aperture.

PARTICIPATION PA IN TRANSPORT OF IONS AND BAP SIGNALING

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Phosphatidic acid (PA) takes special place among signal lipids because it is a key link in the membrane lipid metabolism, on the one hand, and it performs functions of lipid signal molecule, on the other hand. Amount of PA in plant cells elevates transiently under the influence of pathogens, ROS, elicitors, ABA and ethylene. Changes in PA level influence physical properties of membranes and their ability to form vesicles. Some data confirms the ability of PA to transport Ca^{2+} ions through membranes of muscular and nervous cells.

We investigated action of PA with different composition (dioleoyl, dipalmitoyl, from egg yolk) on membrane transport of Ca^{2+}, Mg^{2+} and H^+ using plasma and endomembranes vesicles from maize (Zea mays L.) roots and coleoptiles. Also, of this investigation was to study the influence of BAP on the level of PA.

The 4-day-old etiolated maize seedlings were used. The vesicle preparations were obtained by differential centrifugation and subsequent separation in the PEG/DEX aqueous polymer two-phase system (for isolation of plasma membranes) or in the sucrose density gradient (for isolation of endomembrane fraction). The transport of ions were assayed using Ca-sensitive fluorescent probe Indo-1 (loaded into the membrane vesicles) and Ca^{2+}(Mg^{2+})-sensitive probe chlortetracycline (added to the incubation medium). The proton gradient on the tonoplast vesicles was created by activation of vacuolar H^+-ATPase. The reaction was initiated by the addition of ATP. The increasing of H^+ concentration inside vesicles was registered by the potential density probe acridine orange. The preparation of total pure lipids extracts was isolated on method Bligh and Dyer. The fraction of phospholipids was divided TLC a method Vaskovsky V.E.

Three types of PA were used in experiments; PA was allocated from an egg yolk; PA containing two residues of palmitic acid and PA containing two residues of oleic acid. The greatest effect was observed with PA consisted of two residues of oleic fatty acid. PA including in structure two residues of palmitic fatty acid had the least ionophore properties. The ability of PAs to transport calcium ions across membranes is higher at alkaline conditions than at acidic conditions. We had showed that the ionophore properties of PA are not specific. They are capable to function as membranous transmitting agent not only for Ca^{2+}, but also for Mg^{2+}.

The movement of protons along pH gradient was facilitated by PA as well as FCCP, a well-known protonophore. The highest rate of proton membrane gradient dissipation of tonoplast vesicles caused PA, which consists of two residues of oleic acid. PA with two residues of palmitic acid had the least ionophore activities.

Than we analyzed the influence BAP on change level of PA in cells. We show that the action of BAP on coleoptiles or roots induced the increase of quantity PA and change of fatty-acid composition PA. The received results testify that is probable the action BAP activated PLD. We assume, that PA produced by PLD in plant cells.

Work was financial supported by grants RFBR (no.05-04-49619).

MOLECULAR CHARACTERIZATION OF NOVEL CYTOKININ RECEPTORS IN MAIZE

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Cytokinin (CK) plays an important role for plant growth and development such as cell division, regulation of organogenesis, leaf senescence, chloroplast development and nutrient signaling. We have already identified three genes encoding CK receptors (*ZmHK1*, *ZmHK2* and *ZmHK3*) from maize. Our analyses revealed that the ZmHKs differ in ligand preference, and that the orthologs from maize and *Arabidopsis* show different ligand specificity (Plant Physiol., 2004, 134: 1654-1661).

Here we have isolated three novel genes encoding CK receptors (*ZmHK1a2*, *ZmHK1b1* and *ZmHK1b2*) from maize. *ZmHK1* shows over 90% identity with *ZmHK1a2* and about 60% with *ZmHK1b1* and *ZmHK1b2* at amino acid level. Orthologs of the *ZmHK*s were also found in rice genome, suggesting that they commonly function in monocots. Heterologous expression of each of the ZmHKs in *Escherichia coli* mutant having the Δ*RcsC* and *cps::lacZ* genetic background conferred CK-inducibility of the *lacZ* expression on the bacteria. As is the case with ZmHK1, ZmHK1a2 could respond to *cis*-zeatin at similar extent with *trans*-zeatin in the *Escherichia coli* mutant [Δ*RcsC, cps::lacZ*] assay. These data support our hypothesis that *cis*-type CK is physiologically active in some plant species including maize. ZmHK1b-type receptors showed different ligand preference from those of ZmHK1a-type (ZmHK1 and ZmHK1a2). Another assay with *Saccharomyces cerevisiae sln1* mutant showed essentially same results.

Based on the multiple sequence alignment of CK receptors from maize, rice and *Arabidopsis*, we introduced site-directed mutagenesis into *ZmHK1* and *ZmHK1b2*. The substitutions of specific amino acid residues alter the ligand preference of each receptor. Single amino acid substitution in *ZmHK1* confers constitutive activity to ZmHK1.

Western blot analyses using the antibody against ZmHK1/ZmHK1a2 suggest that subcellular localization of ZmHK1/ZmHK1a2 may be different from those of ZmHK1b-type.

SESSION 2:
Phytohormone crosstalk

SOME INVESTIGATIONS IN THE FIELD OF SALICYLIC ACID MECHANISM

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Salicylic acid (SA) is a phenolic compound that is an important effector molecule in plants. It has been shown to regulate a number of processes, including thermogenesis in aroid plants, the defense response to pathogen and virus attack. There is also evidence for a role of SA in regulating plant responses to some abiotic stresses. SA and H_2O_2 may be the components of one signal system, because under the stress conditions the increase of endogenous SA takes place, which in its turn inhibits catalase and increases H_2O_2 accumulation.

We have investigated the influence of exogenous SA and H_2O_2 upon oxidative processes in cotyledonous leaves of "Fenix" sort of cucumbers. We have isolated cotyledonous leaves from 10-days seedlings and incubated them in a solution containing varying concentrations of SA (0.1, 1, 3 and 5mM) or 10 mM H_2O_2 for 6 hours at room temperature in the first series of the experiment and for 24 hours in the second series. Next we defined the intensity of lipid peroxidation and activity of superoxide dismutase (SOD) in isolated cotyledons (IC). Six hours later we found out 10% increase of lipid peroxidation intensity in IC in solutions SA with concentration 0,1mM. With the concentration of SA 1mM this result enhanced by 20% compared with control IC. 3 and 5mM SA did not change lipid peroxidation compared with control. Under the same conditions the SOD activity decreased with all SA concentrations, not reaching significant values.

Oxidative stress caused by SA is connected with the increase of H_2O_2. In this case IC treated only by H_2O_2 must get analogical damage. In our case 10mM H_2O_2 did not change lipid peroxidation activity, but significantly decreased SOD activity by 15%.

More hard regime of IC seedlings of cucumber cultivation (for 24 hours) confirmed the results obtained with 6 hours IC treatment: the increase of lipid peroxidation activity with concentrations SA 0.1 and 1mM was seen subsequently by 10% and 18%, respectively, compared with control value. With the concentration SA 3mM lipid peroxidation level did not change but with the concentration 5mM decreased by 16% compared with control.

Thus SA solution 0.1mM maximum induces lipid peroxidation activity, but solutions 1 and 3mM maximum reduce SOD activity when IC cucumber are being treated for 6 hours. That may be the result of the fact that SA increases the amount of H_2O_2 not because of SOD activity, but by means of enzymes inactivation, capable to degradate H_2O_2. Factually the ways of receiving and transformation of signals induced by exterior factors and SA are crossed and cause the accumulation of lipid peroxidation products which may be modulators of systemic required resistance. The greater effect of SA was shown in the experiments when the time of IC incubation was prolonged to 24 hours.

IDENTIFICATION AND EXPRESSION ANALYSIS OF *PnACS* GENE FROM *PHARBITIS NIL*

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ACC synthases are the key enzymes regulating biosynthesis of ethylene – one of the plant hormones that controls growth and development. There are many responses regulated via ethylene in reaction to exogenous stimuli. Ethylene is also known to be involved in the regulation of flowering.

A small fragment of *PnACS* gene had been previously identified (GeneBank acc. no. DQ235256). Application of 5'- and 3'- RACE-PCR technique enabled us to obtain the whole sequence of the gene which consist of 2035 bp and its predicted amino acid sequence is 64% homologues to *ACS6* from *A. thaliana*. Subsequently, RT-PCR technique was used to study changes of *PnACS* mRNA level in different light conditions and after IAA treatment. The highest expression level of *PnACS* in cotyledons of *P. nil* growing both in LD and SD condition was observed between 9^{th} and 10^{th} h of 24h cycle. Spectacular increase of the expression level (tenfold) was observed when IAA was applied at the beginning of the inductive night. It also seems that light raised the expression of *PnACS*.

In five-day-old seedlings of *P. nil* growing both in constant light (CL) and transferred from CL to darkness, the expression of *PnACS* occurred in all studied organs (tips, petioles, cotyledons, hypocotyls, roots). The highest expression level took place in roots and the lowest one – in tips. Moreover, mRNA level of the gene was significantly elevated in all organs as early as two hours after IAA treatment in plants cultivated both in CL and transferred to darkness. The top increase of the expression (fourfold) was observed in tips and hypocotyls and the smallest one (twofold) – in roots. Afterwards, mRNA level of *PnACS* was systematically decreasing (4^{th} and 8^{th} h) in all organs to reach the steady-state in 16^{th} hour after IAA application. Furthermore, the expression level of investigated gene was higher in 4^{th} and 16^{th} h than in 2^{nd} and 8^{th} h in plants growing both in CL and transferred to darkness. It could show that the expression of *PnACS* is controlled by circadian clock but additional investigations should be done. Analysis of other genes that involved in ethylene biosynthesis, have to be performed to fully understand their meaning in the process of flower induction.

INVESTIGATION OF POLYAMINES CONTENT AND COMPOSITION IN *GEUM URBANUM* L. UNDER SALT STRESS

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Salinity is one of the negative and most significant factors, which have influence on plant growths and development. Polyamines (PAs) are small ubiquitous and positively charged aliphatic compounds that have an impact on most processes linked to plant growth and development. They play a key role in the control of cell proliferation and cell differentiation and have also a strong influence on germination, flowering, fruit ripening and leaf senescence. PAs also can participate in defence response to different abiotic stress. The investigations of different model objects, for example, *Mesembryanthemum crystallinum* L. and *Thellungiella halophila* Mey. could not give us the clear picture of stress defence mechanisms. In this reason it is very important to investigate the glycophyte plants which cannot tolerate salt stress.

We investigated tolerance of wild herbaceous plant – *Geum urbanum* L. This plant has a wide natural habitat in different climatic zones and able to grow on wastelands. On basis of this enumerate factors we proposed that this plant could have constitutive defence mechanisms and could be resistant to salt stress.

The plants were grown in water culture conditions. In age of 6 weeks the plants were treated with 100, 200 and 300 mM NaCl during 72 hours. We studied the dynamic of free PAs content and composition (putrescine, spermidine, spermine) under this conditions.

It was shown that *Geum urbanum* plants had the high constitutive level of putrescine in roots and low level in leaves. The putrescine level increased only in roots after 24 hours of 100 mM NaCl treatment. We did not observe the stress depended accumulation of this polyamine, how it was shown for non salt résistance plants.

We did not also find the accumulation of others PAs spermidine and spermine nor in leaves nor in roots. Even we observed some decreasing of there content during first to days. After third day under 100 and 200 mM NaCl the spermine level slowly increased both in leaves and roots. This fact and stabile high level of antioxidant enzymes activities in roots could correlate with the beginning of adaptation to salt stress after three days of experiment. Moreover, the roots of *Geum urbanum* seems to more strongly responced to salt stress(100-300mM) then leaves and the defence system in roots effect more strongly in this organ.

Work supported by grant of Presidium of RAS «Molecular and cell biology».

THE PECULIARITIES OF REGULATION OF ENERGY EXCHANGE IN THE CHLOROPLASTS

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Elucidation of self-control principles of the plant organism is based in many respects on understanding the mechanisms of energy exchange regulation. In usual understanding this process includes in itself a number of necessary components, namely: substrate- enzyme-product. However, if we imagine energy exchange as a chemical signal system then an enzyme can be considered as a converter of molecular signals using direct and reverse links of the process control. In this case participation of low molecular phytohormonal substances, the role of which in energy exchange is not understood yet, is possible. Adenylate kinase (EC 2.4.7.3.) of the chloroplasts, which catalyzes the reaction 2 ADP – ATP+ AMP, can be a good model for studying the regulation mechanism of separate biochemical reactions. The control of ATP generation due to the requirements by the effect on the transport of adenine nucleotides insight an organelle is supposed to be in the chloroplasts at the level of functioning adenylate kinase by direct and reverse link of the shuttle type. There is evidence that phytohormones like kinetin and abscisic acid (ABA) can change the rate of phosphorylation. According to the data obtained by us in experiments *in vivo* and *in vitro* kinetin activated adenylate kinase of the chloroplasts, whereas ABA inhibited this enzyme. Different effects of phytohormones on the activity of adenylate kinase of the chloroplast can be possibly explained by the influence on conformational isomerization of the enzyme. Hence, modulation of the activity of adenylate kinase of the chloroplasts by the phytohormones can be one of the elements of transformation of signal generation or regeneration of adenyl nucleotides. In this case the signal system promotes not only changing the kinetics of ATP formation and its consumption, but transferring the energy change from one steady-state level to another. From the view of general biology the regulation of ATP turnover with participation of the hormones and secondary mediators is one of the important mechanisms of the signal system of a cell.

IDEAS WHICH WERE NOT BORN IN TIME

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OXIDANTS. Structure/activity relationships (SAR). All biologically active structures (BAS) contain special fragments named as functional-reactive groups (or descriptors) (http://www.kurchii.h1.ru). The first group of BAS is presented by chemicals that contain an active hydrogen atom (at C- and N-atoms). Chemicals containing these fragments in their structures are activated (i.e. transformed into free radicals) in the reactions of hydrogen atom abstractions. The second group of BAS is presented by chemicals that contain double or triple linkages. These chemicals are transformed into free radicals in the free radical addition reactions. The third group of BAS is presented by transition elements. Activated (transformed into free radicals) BAS initiate free radical chain reactions (i.e. oxidative processes) within the cells. We have implemented a new paradigm in SAR, which permits the high throughput analysis of virtual (biologically active) compounds before their designing and to evaluate biological activity (toxicity) of any chemical without routing daily testing on existing instrumentation.

Chirality of some molecules and its importance to living systems. Usually, only one type (L- or D-forms) of many chiral molecules is presented (used) in biological systems. Our studies from SAR suggest that this phenomenon is caused by the presence of an active hydrogen atom (at the C- or N-atoms) that sterically is not hindered by neighboring radicals and this allows to easy form free radicals in the reaction of the hydrogen atom abstraction.

Ethylene. By chemical properties ethylene (E) is very inert chemical under regular physical conditions, and *in vivo* it may be activated only in the free radical addition reactions. Activated in this reaction E induces oxidative processes first of all in the membraneous structures. At the same time in some experiments E (as all chemicals that contain unsaturated functional groups) imitates antioxidative properties because it catches metabolic free radicals, but this is a temporary effect that subsequently is again transformed into oxidative processes. Hence, E possesses in dual biological function: it is a quencher and source of free radicals.

ANTIOXIDANTS. This group of substances may prevent oxidative reactions and includes important enzymatic and nonenzymatic scavenging systems. Among the high-capacity antioxidants are: (1) Prostaglandins (PGD_2, PGE_2, $PGF_{2\alpha}$); (2) Sterols (cholesterol, testosterone, pregnenolone, 31-norlanosterol, ergosterol, campestanol, sitosterol, stigmasterol, lanosterol, etc.); (3) Carotenoids; (4) Thromboxane B_2 etc.

Abscisic acid. Abscisic acid (ABA) and some its metabolites are the structures behind the special biological function. In the most cases it is a final product from oxidative breakdown of carotenes. Several precursors and metabolites of ABA may act as oxidants or antioxidants. Oxidized ABA may act as an oxidant and activate antioxidative potency of the cells, whereas 1',4'-diol ABA functions as the natural antioxidant.

THEORY OF RECEPTORS IS THE FATAL MISTAKE IN BIOLOGY. Nevertheless, signaling molecules may be presented by fragments of DNA (80-100 bp) named by us as gene keys that are stored within cellular compartments and are liberated during disruption of these compartments. Liberated gene keys are joined to DNA-polymerases and open gene locks (by formation of hydrogen bonds with A-T-nucleotides in the gene lock) that are disposed at the beginning of genes/clusters. Currently receptor proteins are extensively developed in plant and animal systems but questions remain: they really do exist! This is very important because the quantity of natural and synthetic (including drugs, pesticides, poisons, pollutions etc.) BAS greatly exceeds the quantity of genes in the living systems.

UV-B LIGHT EFFECT ON HORMONE-INHIBITOR BALANCE IN ARABIDOPSIS PLANTS

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UV-irradiation influencing on a plant, causes changes in it hormone-inhibitor balance. Hence, the irradiation of plants by ultraviolet results in increased contents of growth inhibitors in the cells, in particular flavonoids and hydroxycynnamic acids. There is a question how the level of some hormones changes in conditions of ultra-violet irradiation. The mutant forms of arabidopsis (*Arabidopsis thaliana* L.), deficient on some enzymes of phenolic biosynthesis, are the good model for studying the answer of a plant on short-wave radiation. Among them two consecutive mutants: tt4 – a mutant on a gene chalcone-synthase and tt5 – a mutant on a gene chalcone-isomerase are interest. We investigated the dependence of accumulation phytohormones abscisic acid (ABA) and indolilacetic acid (IAA) and the common contents of phenylpropanoids in arabidopsis plants of wild type and mutants tt4 and tt5 at two levels of ultra-violet irradiation, 1,37 and 2,11 kJ/m^{2}d.

In conditions of the specified experiment the appearance of wild type and mutants of arabidopsis plants changes appreciablly. Without ultra-violet light the plants of all three types have approximately identical sizes. Irradiation UV-B light results in reduction of the area of leaves and the socket. It is especially appreciable on mutant plants. The leaves area decreases, but the dry weight for a unit of the leaves area increases.

Table. The content of ABA, IAA and a phenolic complex in leaves of mutants and wild type arabidopsis plants at different dozes UV-B light (μg /g dry weight).

UV-B light	Abscisic acid			Indolilacetic acid			Phenolic compounds		
	wt	Tt4	Tt5	wt	Tt4	Tt5	wt	Tt4	Tt5
None	15,9	7,8	4,5	1,2	1,0	0,8	208	163	161
1,37 kJ/m^2 d	14,5	6,1	18,0	1,1	0,8	2,3	272	248	163
2,11 kJ/m^2 d	5,9	13,5	27,0	0,4	2,0	4,0	364	369	170

(the error of measurement is made up 5-7 %)

At affect of ultra-violet light in wild type arabidopsis plants, the contents ABA and IAA decreases, but the level of substances of a phenolic complex inclusing flavonoids and sinapic ethers considerably grows as shown in Table. Mutant plants tt4 at this conditions accumulate as ABA and IAA, and phenolic substances, and the mutant tt5 are increased at the same time with maintenance ABA and IAA and does not change the level of the contents of phenolic compounds. Thus, the effect of low dozes of ultra-violet causes in plants of wild type significant accumulation of substances of the phenolic nature and decrease the level of ABA and IAA. The mutant forms of arabidopsis plants, on the contrary, do not only activate synthesis of the hormones at UV-B light irradiation, but also keeps or raises the level of phenylpropanoids. This makes possible to suppose, that braking of flavonoid synthesis promotes redistribution of aromatic amino acids biosynthesis, probably, at the stage of shikimic way.

THE MECHANISM OF INHIBITION EFFECT OF SALICYLIC ACID ON JASMONATES SYNTHESIS

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We have found the salicylic acid-induced and methyl jasmonate-induced changes of protein set and contents in pea roots with 2D-electrophoresis. Some of these proteins were identified with MALDI TOF MS and MASCOT programm as ABA-responsive protein, L-ascorbate peroxidase, gluthation-S-transferase, PAP-fibrillin, translational elongation factor 1 subunit B, malate dehydrogenase cytoplasmic, NBS-LRR type RGA and so on. We consider, that the most important fact is the salicylic acid-induced disappearance of 12-oxophytodienoic acid 10,11-reductase, catalising one of the final reactions of jasmonic acid (and methyl jasmonate) synthesis. It can explain the mechanism of the earlier revealed inhibition of jasmonates synthesis (and a part of jasmonate-inducing proteins) by salicylic acid.

EFFECT OF RED LIGHT ON THE IAA TRANSPORT IN THE SUGAR BEET SEEDLINGS

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The red light mediates a variety of physiological processes, including the transport of many substances through the membranes. Among the substances which transport may be influenced by irradiation is phytohormone auxin. To verify this assumption we studied the effect of red light and far-red light on the auxin transport in the sugar beet seedlings (*Beta vulgaris* L.). In this study we have used labeled (3H-IVA) indole acetic acid (IAA). Etiolated sugar beet seedlings (15 mm) were cultivated on agar-cultured medium.
Etiolated seedlings were divided into three sets of experiments and exposed to red light and far-red light for 10 min. The first set of seedlings was exposed to red light and the second one to far-red light. The third set of the seedlings was exposed to red light and immediately to far-red light.

Seedlings from all sets were divided into two (upper and lower) parts. The upper part of seedlings was treated with 1μM (3H-IVA) IAA. Following 10 min after the treatment the upper part of seedlings was cut and IAA from this part was extracted (fraction 1 of IAA). Intact seedlings by lower non-labeled part were immerged into agar medium in the dark box for 24 h. After 24 h maintaining on agar medium upper part of seedlings was deleted and IAA from this part was extracted (fraction 2 of IAA). Fraction 3 of IAA was received from lower part of seedlings. IAA in all fractions was identified by thin layer chromatography. IAA from plates was extracted and its radioactivity was measured.
It is found that in the cutting seedlings exposed to red light the content of IAA (evaluated by radioactivity) was higher from 15% up to 55%. Far-red light do not influence IAA transport in the seedlings. Also in the experiments where seedlings were exposed to red light and re-exposed to far-red light the stimulatory action of red light was abolished.
We have studied the binding of IAA to membranes. It is shown that membrane fraction from the seedlings exposed to red light and from the seedlings which seeds were irradiated by red light contained up to 2 fold higher of labeled IAA in comparison to ones non-exposed. These effects were depended on the time of irradiation.
It is concluded that irradiation of the sugar beet seeds and seedlings by red light and far-red light influences IAA transport and its conjugation with cellular substances.

PHASEOLUS VULGARIS L. PRIMARY LEAF GROWTH AND ENDOGENOUS PHYTOHORMONES UNDER DROUGHT AND THE EFFECT OF SEEDS TREATMENT WITH ABA

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Plant tolerance to water stress and ability to survive under drought depends on the organism age. The youngest plants are more sensitive to stress but they more easy acquire resistance. Plants treatment with growth regulators can change their tolerance to water stress and recovery after one. The aim of this presentation is to reveal the possible mechanisms of these phenomena at the hormonal level.

Phaseolus vulgaris L.seeds were grown in soil in controlled condition after 3 h imbibition in water or in ABA solution (10^{-6} M). Endogenous phytohormones and anatomy of primary leaf at the stages of maximum mitotic activity (5-th d after germination), cell elongation (9-th d after germination) and growth termination (14-th d after germination) under water deficit were studied by HPLC, bioassays and microscopy methods. Water deficit was induced by cessation of watering during 2 days at all these stages.

Highest levels of zeatin, zeatin ribozide and free gibberellin-like substances (GLS) were determined at the beginning of control plants leaves development. Free cytokinins disappeared and IAA level declined whereas content of zeatin-O-glucozide and ABA increased at the growth termination stage.

Alterations in phytohormones balance were determined under drought at all studied stages. Cell division inhibition and as a consequence decreasing in leaf area were observed as a result of phytohormones disbalance. At the same time the increase of cell thickness and density of palisade tissues took place. The most considerable changes in phytohormones under drought were shown at the stage of maximum mitotic activity of leaf cells: zeatin content decreased 5 times, IAA – 2 times, GLS activity – 4 times. When drought was created at the stage of leaf cells elongation alterations were less essential but 2 fold enhancement in ABA level was shown. *Ph. vulgaris* leaves were the less sensitive to water deficit at the stage of growth termination. Changes in free hormones content were not detected but levels of zeatin-O-glucozide and bound IAA and ABA increased more than 2 times. Phytohormones disbalance was found during long time after rehydratation especially if drought was created at the stage of maximum rate of meristem cells division.

As a result of seeds imbibition in ABA solution increase in leaf thickness and area, formation of the greater stomata amount were observed. Chloroplasts amount did not change. Enhancement in endogenous free and bound IAA and ABA, zeatin, zeatin ribozide, zeatin-0-glucozide, as well as increase in free and bound GLS activity was shown during leaf growth and development. When seeds were treated with ABA solution the tendency to decreasing in stimulating hormones content under drought saved but differences between control and experimental leaves characteristics were less essential and at the stage of leaf maturing they became unnoticed.

Thus, content of zeatin, zeatin ribozide, IAA, ABA and GLS activity declined whereas amount of free ABA increased in *Ph.vulgaris* primary leaf under water deficit. The most considerable changes in phytohormones content under drought were determined at the earlier stages of leaf development. Therefore, the mechanism of plant sensitivity to water stress can be assumed to be connected with reactivity of hormonal system which obviously changes during ontogenesis. Treatment with ABA did not prevent negative effect of water deficit completely but promoted the growth processes normalization.

THE POSSIBLE ROLE OF NITRIC OXIDE AND SUPEROXIDE IN AUXIN SIGNAL TRANSDUCTION

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One of the most relevant problems of the modern plant physiology is the deciphering of molecular mechanisms of the hormone signal transduction. During last years the participation of some small oxygen derivatives like nitric oxide (NO) and reactive oxygen species (ROS) in pro- and eukaryotic signaling was revealed. In particular it was shown that plant hormone auxin promotes the formation in plant tissues some free radicals as nitric oxide, superoxide (O_2^-) and hydroxyl ($HO^{\cdot}$). These small oxygen derivatives were able to mimic some auxin effects on rhizogenesis, cell division and cell elongation (Schopfer et al., Planta, 2002; Correa-Aragunde et al., Planta, 2004; J.Exp.Bot., 2006; Hu et al., Plant Physiol., 2005). However it remains unclear whether these small molecules take part in auxin signal transduction or act on some later stages.

To clarify this, we have accomplished experiments with wild type and transgenic Arabidopsis seedlings. Using cell-permeable probe DAF-2DA for nitric acid imaging, we have detected the NO accumulation in Arabidopsis roots shortly after auxin treatment. This allowed us to suppose the direct influence of auxin on enzyme(s) generating nitric oxide. Next we have extended our studies on transgenic *DR5*::*GUS* Arabidopsis expressing the reporter gene *GUS* under control of the auxin-sensitive promoter *DR5*. As additional model system we have used transgenic *FER*::*GUS* Arabidopsis expressing the same reporter gene, but under control of the NO-sensitive promoter from *AtFER1* gene. The expression level of transgenic constructions was determined by means of quantitative fluorometric assays.

It was shown that different NO donors (NOR3, SNP, S-nitrosothiols) significantly enhanced the expression the *DR5*::*GUS* construction in seedlings. By contrast, NO scavengers (cPTIO, hydroxycobalamin) as well as NO-synthase inhibitor L-NNA markedly inhibited the auxin effect on *DR5*::*GUS* expression. This inhibition could be alleviated by NO-donor SNP at low concentration.

Auxin (10 μM) increased the expression of the *FER*::*GUS* construction 3-6 fold. L-NNA suppressed this auxin effect.

Also we have studied the influence of different forms of reactive oxygen species (ROS): superoxide anion, hydroxyl radical, hydrogen peroxide and peroxynitrite, on *DR5*::*GUS* expression. It was shown that only superoxide anion induced significantly the expression of this transgenic construction. Other forms of ROS, on the contrary, suppressed the auxin effect, except hydrogen peroxide which was almost ineffective in this system. These results were corroborated using specific scavengers of free radicals. $CuCl_2$, a scavenger of superoxide, reduced the degree of auxin-induced transcription of *DR5*::*GUS* construction, whereas thiourea, a scavenger of peroxynitrite, reinforced the auxin effect.

$ZnCl_2$ at low (0.3 mM) concentration inhibiting the activity of NAD(P)H-oxidase (known to be able to produce superoxide anion) also markedly suppressed the auxin effect.

Taken together, these results give an experimental basis for the suggestion that nitric oxide and superoxide anion are tightly linked to the primary cellular response to auxin, on the level of gene expression. It is not excluded that nitric oxide and/or superoxide anion take part in intracellular transduction of the auxin signal. However the deciphering of the precise role and site of action of these small molecules needs further investigation.

PHYTOHORMONES IN INTERNODES OF DIFFERENT DIFFERENTIATION DEGREES IN FERTILE THALLI OF *CHARA CONTRARIA* A. BRAUN EX KÜTZ. (CHAROPHYTA)

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We investigated the phytohormonal balance in internodes of different differentiation degrees in the fertile thallus of the freshwater alga *Chara contraria* A. Br.: VIII top young, VI medium, IV and II (from rhizoids) lower mature internodes with nodes and their components – the cell wall of the central cell of the internode with cortex, cytoplasm with organelles, and nodes detached from the whole internode. Identification of phytohormones (IAA and ABA) was performed using a HPLC method. During the growth, the levels and interrelations of phytohormones changed in internodes of different degrees of differentiation. Bound forms of IAA prevailed over free forms in internode IV. The highest level of ABA was registered in internode VIII, which is a part of the apical portion of the main shoot. In internodes from the top and medium parts of shoots of the fertile thallus of the alga, free forms of ABA prevailed, while bound ABA forms were dominant in the lower parts. The lowest levels of both forms of IAA and ABA were observed in the lower mature internode II. As to contents of phytohormones in structural elements of the VI medium internode and both IV and II lower internodes, we demonstrated that the highest contents of both forms of IAA were peculiar to the cytoplasm of the central cell of internode VI. Free forms of that hormone quantitatively dominated among its all revealed forms in cytoplasm of all internodes studied. Highest contents of both forms of IAA were found in components of the upper young metemeres: in the cell wall of the central cell of internodes with cortex, protoplasm with organelles and in nodes; the lowest content was observed in lower senescent ones. In cytoplasm with organelles we observed the same tendency of ABA allocation, but in the cell wall of the central cell of the internode with cortex and in nodes the pattern was inversed. We revealed the presence of a gradient of allocation of different forms of IAA and ABA along a the vertical axis in whole internodes of different age and their components, which probably means that body the process of growth of internodes of the main shoot of *Chara* is under control of a balanced phytohormonal complex.

THE ROLE OF ABSCISIC ACID AND ETHYLENE IN THE REGULATION OF FLOWERING IN *PHARBITIS NIL*

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Ethylene is a strong inhibitor of flowering in short day plants (SDP). Whereas the data concerning the involvement of abscisic acid (ABA) in the regulation of flowering of plants are not definitely clear. ABA can both stimulate and inhibit flowering of SDP *Pharbitis nil*.
The dual role of ABA in the photoperiodic flower induction of *Pharbitis nil* was shown in our experiments throughout exogenous applications and endogenous level determination of the hormone in cotyledons under different light condition.
Application of ABA on the cotyledons during the inductive night (16h) inhibited the flowering. However, ABA application on the cotyledons or the shoot apices during the subinductive 12h-long night results in the slight stimulation of flowering. The NDGA, an abscisic acid biosynthesis inhibitor applied on the cotyledons of 5 day-old seedlings during the inductive night (16 h) inhibited formation axillary and terminal flower buds.
During 16h-long inductive night the level of ABA slightly changed but when the inductive night was interrupted by irradiation with 10 min pulse of red light (R) given in the half of the night, the endogenous ABA level in cotyledons clearly decreased. Lower levels of ABA was observed in seedlings treated with NDGA during the inductive night. The level of ABA decreased also after ethylene treatment during the inductive night.
These results suggest that ethylene may inhibit flowering in *Pharbitis nil* through the decrease of ABA level. Confirmation of this possibility may be the fact that simultaneous treatment of induced seedlings both with ethylene and ABA strongly reverse the inhibitory effect of ethylene on flower induction. Our results also suggest an important role of ABA in photoperiodic induction of flowering in *Pharibitis nil* seedlings.

SODIUM NITROPRUSSIDE, NO-DONOR, AS A TOOL FOR INVESTIGATION OF NITRIC OXIDE SIGNALING VIA MICROTUBULES IN *ARABIDOPSIS THALIANA* ROOT CELLS

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During last years novel and diverse functions are being attributed in plants to nitric oxide (NO), a diffusible second messenger. They extend from cell growth and developmental processes to pathogen defense and stress tolerance, including stomatal closure, seed germination, root development, expression of defence-related genes and programmed cell death (Neill et al, 2003). Most of these processes can be based on signalling role of microtubules (MTs) (Blume et al., 2006). There are experimental evidences that plant α/β-tubulin undergoes to post-translational modifications, but very common for animal MTs α-tubulin nitrotyrosination is not yet deeply observed. Potential role of α-tubulin nitrotyrosination on plant tubulin structure we analysed earlier (Blume et al., 2005), whereas its functional impact on plant MTs organization has not been investigated. The goal of this study was elucidation of NO-donor sodium nitroprusside (SNP) effects on MTs organization in plant cells.

Arabidopsis thaliana line expressing GFP-MBD (MAP microtubule binding domain) was used in this research. Four-days-old seedlings of *A. thaliana* were treated with different concentrations of SNP (10, 100, 250 and 500 mkM) during 4, 12, 24 and 48 h. GFP-labeled MTs were visualized *in vivo* using confocal laser scanning microscope LSM 510 META (Carl Zeiss, Germany). The obtained results indicate that SNP treatment during 24 h in all tested concentrations leads to primary root growth promotion, while its treatment during 48-72 h resulted in significantly decrease of root length. This effect of SNP on primary root growth is in accordance with its influence on general root morphology. After 24 h of SNP (250 and 500 mkM) treatment the maturation zone (zone of root hair formation) was considerably enlarged in comparison with control. SNP treatment during 48 h led to significant reduction of cell growth in elongation zone (roots ceased to elongate), whereas cells differentiation was significantly stimulated, that resulted in induction of new root hairs formation.

At the same time significant effects of tested concentrations of SNP on MTs organization in different root cell types were observed. It was established that epidermis cells in elongation zone were the most sensitive to SNP action, where considerable disturbances in MTs orientation were observed. It was shown that 24 h treatment with 250 mkM SNP leads to change the native MTs orientation from transverse to oblique or even longitudinal in epidermis cells of elongation zone. SNP in 500 mkM concentrations led to MTs randomisation after 4 h treatment, whereas 24 h treatment resulted in change of MTs orientation from transverse to longitudinal in epidermis cells of elongation zone. It was found that SNP disrupts cortical MTs orientation in a time- and dose-dependent manner. We suppose that SNP can cause new root hairs initiation and formation through induction of cortical MTs destruction. Since early it was shown that auxin is essential factor for cortical MTs randomization and root hair initiation (Takahashi et al., 2003), we assume that NO-donor could take part in signalling cascade downstream of auxin. This suggestion corresponds also to the data obtained recently on tomato (Correa-Aragunde et al., 2006), where it was demonstrated that NO modulates the expression of cell cycle regulatory genes in tomato pericycle cells and that NO is required during the early stages of lateral root development.

Neill SJ, Desikan R, Hancock JT. Nitric oxide signalling in plants. New Phytol 2003;159:11-35.

Blume YaB, Nyporko A, Demchuk O. Nitrosination of plant α-tubulin: potential mechnisms of influence on cellular processes. BMC Plant Biol 1995; 5:S26.

Blume YaB, Nyporko A, Yemets A. Nitrotyrosination of α-tubulin: structural analysis of functional significance in plants and animals. Cell Biology and Instrumentation: UV Radiation, Nitric Oxide and Cell Death in Plants (Eds Ya. Blume, D. Durzan and P. Smertenko), NATO Science Series, IOS Press, 2006, p. 325-333.
Correa-Aragunde N, Graziano M, Chevalier C, Lamattina L. Nitric oxide modulates the expression of cell cycle regulatory genes during lateral root formation in tomato. J Exp Bot 2006; 57(3):581-588.

SESSION 3:
Brassinosteroid signaling

PROTECTIVE EFFECT OF BRASSINOSTEROIDS ON SEEDS WITH DIFFERENT MATURE STATUS UNDER UNFAVORABLE STORAGE CONDITION

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Brassinosteroids (BRs) as steroidal plant hormones were determined into different parts of plants but the most amount of them was found in the pollen and immature seeds (Khripach *et al*, 1999). In seeds they may regulate the processes of dormancy and germination. BRs together with GA are needed to overcome inhibition effect of ABA and to stimulate germination (Steber *et al*, 2001). Little is known, however, about influence of BRs on seed deterioration under unfavorable conditions of storage. The aim of our work was to study the BRs influence on the germination of seeds with different physiological quality determined by maturation level and storage conditions.

A commercial seed lot of white cabbage (*Brasicca oleracea* L.) was sorted on maturity, using a chlorophyll fluorescence sorter *(Sakata,* USA*)*. Sorted seeds have been treated by infusion of epibrassinolide (Eb) or homobrassinolide (Hb) with using benzene as carrier and aged at 86% of moisture content for 3 days. It is important to notice that the treatment with benzene didn't result to increasing seed moisture content before aging.

It was reported about wide variability of maturation degree within single seed lots of some crops: Chlorophylls may be used as a marker for cabbage seed quality (Jalink *et al.*, 1999). In our research chlorophyll content in cabbage seeds ranged from 4 to 14 μg/g of dry matter. Carotenoid concentration changed dependently of chlorophylls: less-mature seeds had 17 μg/g lutein and 8 μg/g β-carotene, in more-mature seeds that values decreased to 8 and 2,2 μg/g accordingly.

Less-matured cabbage seeds showed a slower germination rate and developed a higher amount of abnormal seedlings. Seed aging resulted to faster decreasing the quality of less-maturated seeds in comparison to mature ones. Under stress condition of seed storage the degradation Chl content from 4 to 3 μg/g and the increasing lutein content from 8 to 10 μg/g were observed. As Eb and Hb infusion into seeds reduced the rate of deterioration, since aged seeds pre-treated with Eb and Hb gave 64% and 65% normal seedlings compared to 50% in control. At that some increasing carotenoid content under influence of BRs treatment has been observed in deteriorated seeds. That content increased on about 30% in less-mature seeds and about 5% in mature seeds.

Thus, the synthetic analogs of BRs such as Eb and Hb infused into dry seeds had prevented the rate of seed deterioration under unfavorable conditions of accelerated aging and had long-time influence on growth, development and tolerance of seedlings. The efficiency of treatment with BRs depended on seed physiological quality: the lower tolerance to unfavorable conditions of accelerated aging, the higher effect of exogenous treatment. The obtained date allowed proving the commercial application of synthetic BRs analogs for quality of seeds under long-storage.

Acknowledgements: The authors thank Prof. Vladimir Khripach for supply of epibrassinolide and homobrassinolide.

EFFECTS OF 24 EPIBRASSINOLIDE AND PLANT GROWTH STIMULATORS ZIRCON AND EPINE-EXTRA ON GROWTH AND DEVELOPMENT OF POTATO *IN VITRO*.

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One of key stages in preparation of elite seeds of potato is virus removal by apical meristem cloning. This process is time-consuming. The goal of this work is to evaluate effects of growth stimulators (24 epibrassinolide, Zircon and Epine-extra) on potato plants *in vitro*. Experiments were conducted on v. «Ilyinsky» potato cuttings. Control plants were cultivated in standard Murashige-Skoog medium without hormones. Experimental specimens were cultivated on Murashige-Skoog medium with addition of 24 epibrassinolide (10^{-8} M, 10^{-9} M and 10^{-11} M), commercially available compound Zircon (1 ml/L, 0,33 ml/L and 0,2 ml/L) and commercially available compound Epine-extra (1 ml/L, 0,5 ml/L and 0,2 ml/L).
Growth was analyzed by measurement of stems and roots length and biomass increase on weekly basis. It was shown that Epin at all tested concentrations, epibrassinolide at 10-8 M and Zircon at 1 ml/L inhibited plant growth.
Epibrasinolide and zircon demonstrated concentration dependent effect. Nanomolar concentration of epibrassinolide (10^{-11} M) and Zircon (1 ml/L) accelerate growth of both roots and stems by 30%. Epibrassinolide at 10-9 M and Zircon at 0,33 ml/L have no effects on root system but accelerate stem growth by 30%.
Results indicate that 24 epibrassinolide and Zircon at low concentrations can be recommended for *in vitro* plant cultivation. Growth inhibition by Epin-extra is possibly caused by the detergent and ethanol, which are present in the compound, and can be toxic when added directly in to culture medium.

INFLUENCE OF BRASSINOSTEROIDS COMBINED WITH PESTICIDES ON GROWTH AND DEVELOPMENT OF *TRITICUM AESTIVUM* L. PLANTS

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Endogenous system of regulation together with the using of physiological active substances is a basis for management of crop plants. However against a background of high level of phytopathogenic infections application of only growth regulators without protective substances is not be able to provide the good crop productivity. It is necessary to develop the technologies for their combined application.

The aim of this work was to study the effect of seed coating with epibrassinolide and homobrassinolide as the synthetic analogs of natural plant hormones in the complex with fungicide thiram, commercial fungicidal preparation 'Vintsit' ("Ceminova" Denmark, includes the mixture of tiabendazol and flutriafol) and insecticide imidacloprid. The peculiarities of growth and development of spring wheat plants were studied depending on seed treatment. Seed coating compositions were developed based on polymer polyvinylacetate dissolved in organic solvent.

Seed germination tests in laboratory conditions shown the effect of some declining in germinability under the influence of fungicide and insecticide treatment. The adding of epibrassinolide to protective composition did not result to sufficient elimination of that inhibitory influence. But the using of epibrassinolide and homobrassinolide mixture (50:50) resulted to good seed germination and following normal seedling development. The root system length of treated seedlings was equal to ones in untreated control but the dry matter content increased on 14 %. As a result the seedling index shifted towards to root system part.

In field experiences the analysis of morphophysiological parameters of spring wheat plants has been done at phase of field emerging (11 on Zadoks scale), tillering (23-24 on Zadoks scale) and full ripeness. Seed coating with fungicide, insecticide and mixture of epibrassinolide and homobrassinolide resulted to increasing the amount of seedlings per square meter, stimulating the process of shooting and developing the more vigorous plants with higher value of dry matter. Also the leaves of plants grown from treated seeds had higher content of photosynthetic pigments.

As a result of seeds coating with composition from 'Vintsit', thiram and imidacloprid the efficiency of plants has been improved. The number of productive shoots, weight and quantity of grains per one plant and weight of 1000 grains was increased in comparison to control.

Combining of brassinosteroids with protective compounds in composition for seed coating is able to promote the elimination of inhibitory effect of pesticides on seedling emerging and development. At that the mixture of epibrassinolide and homobrassinolide had shown the more perspective results and may be recommending for commercial seed treatment as part of composition together with fungicides and/or insecticides.

Acknowledgements: The authors thank Prof. Vladimir Khripach for supply of epibrassinolide and homobrassinolide.

EFFECT OF COLD STRESS AND 24-EPIBRASSINOLIDE ON LIPOXYGENASE ACTIVITY IN MAIZE SEEDLINGS

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Plants have evolved complex signaling pathways to coordinate responses to developmental and environmental information. Brassinosteroids (BRs) are a class of steroid hormones essential for normal growth and development in plants. The lipoxygenase (LOX) signaling system is involved in cell response to various pathogens, mechanical injuries, elicitors, and some other primary signals. The interaction among the LOX pathway and BR-signaling remains unclear.

The modulation of the activity of maize seedlings LOX by cold stress in presence 24-epibrassinolide (EBR) has been investigated. LOX activity was measured *in vitro* after incubation of seedlings with 0,01-1 μM EBR. Dark grown, 5d-old maize seedlings were exposed to 5° C for 24 h, then LOX were extracted from mesocotyl by the method of Poca et al., 1990. LOX activity was determined using linoleic acid as substrate at pH_{opt} 6,0 and 7,0 in presence and absence 0,02 % Lubrol PX, respectively. We show that after cold stress LOX activity was higher in EBR-treated than in untreated seedlings. The level of oxygenated linoleic acid at pH_{opt} 6,0 and 7,0 by LOX from seedlings grown in the presence of 1 μM EBR increased more than 5- and 10-fold respectively than in control seedlings grown in the absence of the compound. As LOX activity increased upon BR application in cold stress, it provides a potential link between BR-action and the level of oxygenated derivatives of polyenoic fatty acids formed during LOX reactions.

The possible pathways of involvement LOX metabolites in formation of cell response to BRs will be discussed.

This study was supported by Fundamental Researches State Found grant № Ф14/247-2007.

EFFECTS OF 24 EPIBRASSINOLIDE, ZIRCON AND EPINE ON EARLY DEVELOPMENT OF SUNFLOWER, WHEAT AND LUCERNE.

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Biostimulating compounds are widely used to promote earlier activation of metabolic processes and to accelerate development of plants. However the mechanisms of their activity particularly at cellular level are still poorly understood.
In present work effects of 24 epibrassinolide (10^{-8}, 10^{-9}, $5*10^{-10}$ and 10^{-11} M), Zircon (1, 0,33 and 0,2 ml/L) and Epine-extra (1, 0,5 and 0,2 ml/L) on early development of sunflower (*Heliantus annuus* L.), wheat (*Triticum aestvum* L.) and lucerne (*Medicago sativa* L.) were studied. Control specimens were couched in water. Biomass accumulation and length of roots and stems were measured. Measurements were performed at 3,5, 9 and 14th days of cultivation. Cell cycle progression and proliferation were analyzed in root meristem samples.
It was shown that 24 epibrassinolide is effective for all species tested. Best result for wheat was obtained with 10^{-8} M (110% increase in biomass production); for sunflower $5*10^{-10}$ M (200% increase); for lucerne 10-11 M (more than 180% increase). Cell cycle analysis of wheat root meristem demonstrated accumulation of G2-cells. Mitotic index increases.
Zircon was effective in 1 ml/L concentration for lucerne and sunflower. Lucerne demonstrated increase in biomass production for 170%, roots and stems length increased by 30% and 18% respectively. Sunflower seedlings biomass increased by 40%. Lucerne demonstrated accumulation of G2-cells and increase of mitotic index in root meristem.
Epine in high concentration stimulated growth of sunflower and wheat (adding 30% and 70% respectively) but had no effect on lucerne seedlings.
Thus 24 epibrassinolide, Zircon and Epine-extra are recommended for agricultural applications after determination of most effective compound and concentration for particular plant.

THE EFFECTS OF BRASSINOSTEROIDS ON THE POLYPHOSPHOINOSITOL METABOLISM AND PLANT SEEDS GERMINATION UNDER COLD TEMPERATURE.

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The capability of most higher plants to tolerate environmental conditions strongly depends on their developmental stage. Environmental factors can effects on many developmental processes. Seedling emergence is comprised of both germination and early seedling development. Because seedlings are particularly sensitive to cold stresses the planting of most species in temperate regions is dictated by soil and air temperatures in early spring. The problems of seeds germination at low temperatures and increasing of plant tolerance to cold at early stages of development are great importance for agriculture. Plants must adjust their physiology to changes in environmental temperature conditions in order to prevent damage and ensure survival. A better understanding of physiological and molecular mechanisms of cold response could provide targets for manipulation of susceptible species leading to higher yields, longer growing seasons and larger growing areas of crop plants. Some phytohormones can modify seeds germination under unfavorable temperatures. Little is known about role of brassinosteroid in the regulation plant seeds germination under low temperatures.
We investigated the effects of 24-epibrassinolide (EBL) (10^{-6}; 10^{-7}; 10^{-8} M) on seeds germination under low temperature and inositolphospholipids composition of plant coleoptiles. Seeds and seedlings of rape (Brassica napus) and maize (Zea mays L.) were used for experiments. Plants were grown in the dark at 5°C (rape) and at 10°C(maize). For the inositolphospholipids analysis, coleoptiles were placed in flasks containing [^{33}P]orthophosphate for 16h. It was shown, that the EBL to increase germination rape and maize at low temperatures. The analysis of phospholipids showed that the level of radioactivity in inositolphospholipids - PIP and PIP_2 was higher under low temperatures in EBL treated plants, in comparison with plants, which were growth under cold without EBL. Our results suggest, that EBL can activate phosphatidylinositol kinases and maintenance more high level of inositolphospholipids under low temperatures.

This study was supported by Fundamental Researches State Found grant № Ф14/247-2007.

SYNTHESIS AND STUDY OF NOVEL OF BRASSINOSTEROID DERIVATIVES

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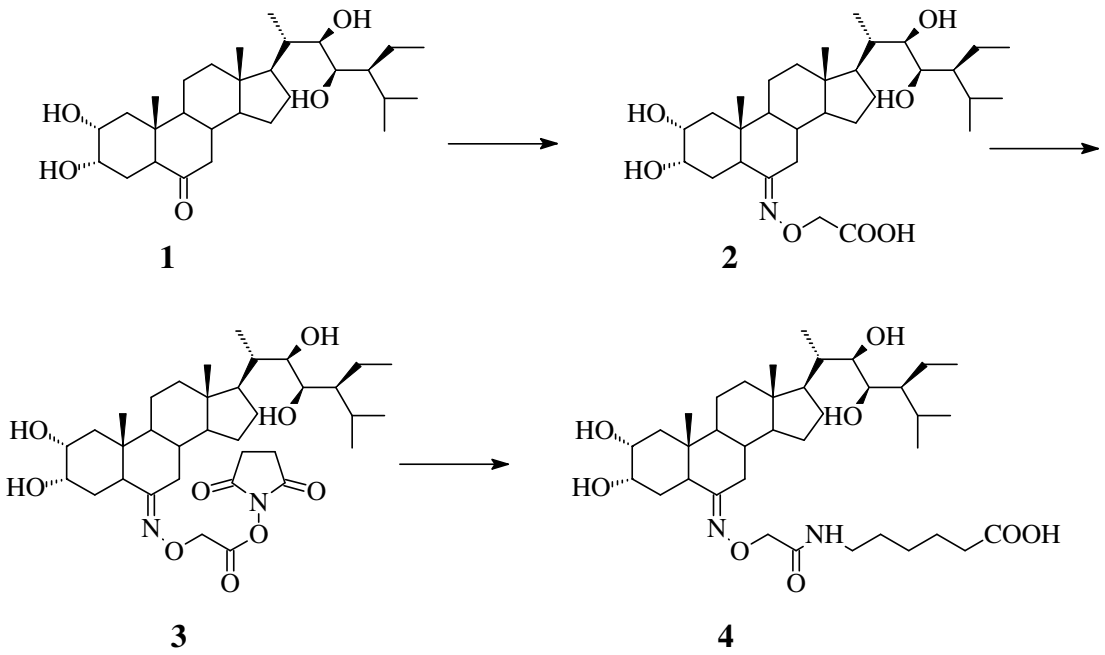
Brassinosteriods (BS), a new group of phytohormones, are plant growth regulators and adaptogens[1]. In the course of our work on BS synthesis we prepared novel 6-oxime derivatives of 28-homobrassinosteroid. The effective synthesis of the title compounds based on the reaction of 28-homocastasterone **1** with (aminooxy)acetic acid. The formed carboxymethyloxime **2** *via* active N-succinimide ester **3** was further converted into derivative **4** by treatment with ε-aminocaproic acid.

Evidence for the structures **2**-**4** was obtained by spectral methods; details of preparation and identification procedures will be discussed.



Physiological activities of the compounds synthesized have been studied *using Rhododendron maximum* as a model plant. The growth stimulating effect of the synthesized derivatives of brassinosteroid oximes has been found.

1. Khripach V.A., Zhabinskii V.N., Ae de Groot. Brassinosteroids - A New Class of Plant Hormones. Academic Press, 1999, 456 p.

BRASSINOSTEROIDS, RESISTANCE AND PRODUCTIVITY OF WHEAT

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A considerable amount of experimental data was amassed in the field of physiology, biochemistry and application of brassinosteroids. They are concerned with many aspects of distribution, biosynthesis, synthesis and effect peculiarities of these substances on plants. Experiments with many plant species have shown that their treatment with brassinosteroids greatly increases the yield. Here not only brassinosteroid preparation use, but also conditions of their application, including concentration, time and method of treatment, as well as meteorological conditions during plant vegetation are of great importance in regulating fruit formation. As to a protective action of brassinosteroids, this research field was initiated by us above 20 years ago. Over this period antibiotic activity of these substances was revealed, the outlook for their use for barley plant protection was shown, with the method for protecting the crop against leaf diseases being worked out and the mechanism of their immunizing effect being disclosed.

The object of the given research was spring wheat of cultivar Rostan grown under field trial conditions. The aim of the work was to ascertain the degree of an increase in the resistance to phytopathogenic fungi and in crop productivity by treating plants with 24-epibrassinolid preparations (epibrassinolid, homobrassinolid and their mixture). Plant treatment was performed by the spraying method at 5 mg/ha dose at the complete tillering stage. Application of the preparations was revealed to favor improvement of the phytosanitary state of sowings already at the shooting stage. The degree of plant damage (primarily with mildew) decreased, on the average, by 5-10% in all the variants as against the control. Homobrassinolid treatment reduced this parameter by a factor of 5. Plant damage was slight at the heading stage and was practically at the level of the control in the variants. Such a pattern remained over the whole period of subsequent observations. The highest effect was noted under homobrassinolid treatment of plants. The treatment did not exert any effect on the qualitative pathogen composition. As a result of the analysis, the following pathogens were identified: Helminthosporium sativum, Alternaria tenuis, Erysiphe graminis, Fusarium spp., Puccinia triticina, Septoria tritici, Ophiobolus graminis and Ascochyta graminicola. The prevailing pathogen was Erysiphe graminis.

The increase in the plant resistance under the effect of the preparations tested resulted in the increase in the wheat productivity, on the average, by 20%. Homobrassinolid, which increased productivity by 32%, proved the most effective. So, if the grain productivity was 26.9 centners per hectare in the control experiment, then it was 35.4 centners per hectare in the variant with homobrassinolid application.

Thus, the conducted research on studying the brassinosteroid effect on spring wheat plants, cv.Rostan, has shown that the used preparations "epibrassinolid" and "homobrassinolid", as well as their mixture exerted a positive effect on the disease resistance of the crop that made an opportunity for realizing its cultivar productivity potential under soil-climatic conditions of conducting an experiment.

EFFECTS OF EPINE AND GEZAGARD ON THE PHOSPHOLIPASES ACTIVITIES IN VARIOUS WEED SPECIES

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Brassinosteroids (BR) are sterol phytohormones that ubiquitously distributed throughout the plant kingdom. BR regulate the expression of numerous genes, contribute to the regulation of cell division and differentiation. BR have a broad spectrum of activities that have a positive effect on the quantity and quality of crops and increase plant resistance to stress and phytopathogens, and can be used as a substitute for some traditional pesticides.

Influence of various concentrations of BR epine and herbicide gezagard on pattern of phospholipase status (ratio of phospholipase activitites) in three species of weed in agricultural areas was studied. Activities of key enzymes of membrane phospholipid catabolism: phospholipase D (PLD), C (PLC) and A_2 (PLA_2) were evalueted in tissues of *Chenopodium album* L., *Galinsoga parviflora* Cav. and *Thlaspi arvense* L. It was found that gezagard and epine affected phospholipase activities and their modulation effect depended on the species of weed, as well as mode and time of treatment. The mechanisms of gezagard and epine effects on phospholipid metabolism in plant cell are discussied.

THE INFLUENCE OF EPIBRASSINOLIDE IN THE COMPLEX WITH FUNGICIDAL COMPOSITION 'VINTSIT' ON THE PHYSIOLOGICAL AND BIOCHEMICAL FEATURES AND THE PRODUCTIVITY OF DIFFERENT BARLEY GENOTYPES

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To decrease the phytotoxic influence of fungicides especially on the first phases of plant development using the various growth regulators is perspective. In our experiments the effect of seed coating with epibrasinolide as the analog of natural plant hormone in the complex with commercial fungicide composition 'Vintsit' has been studied.

Four barley genetic forms (varieties Roland, Zazersky-85 and their isoplasmatic lines) were used as the models differing on sensitivity to physiologically active substances. 'Vintsit' includes the mixture of fungicide tiabendazol (2,5%) and fungicide flutriafol (2,5%). It was applied to barley seeds by coating (2 liters/ton of seeds). Epibrassinolide was added to coating composition in concentration of 10^{-5}% (10 liters/ton of seeds).

The mechanism of phytotoxic action of fungicide 'Vintsit' was shown during seedling development on phase of first true leaf. Fungicide inhibited the linear growth and the biomass accumulation of seedling leaves in all genotypes but with different degree. At that the activation of free radical oxidation and some reduction of chlorophyll *a* content were observed. In more sensitive isoplasmatic genotypes with Roland's nucleus the delay of growth was accompanied by the decrease of content of ready soluble and structural proteins, the inhibition of photochemical activity of chloroplasts and the accumulation of water-soluble carbohydrates. In more resistant isoplasmatic genotypes with Zazersky's nucleus the fungicide mainly reduced the content of ready soluble proteins.

At an average degree of development of root rot 'Vintsit' did not influence essentially on the mass of 1000 grains in all barley genotypes and on the mass of grains from one plant in genotypes with Roland's nucleus.

Epibrassinolide inhibited the negative effect of fungicide on the linear growth and the biomass accumulation of seedlings, keep up the contents of ready soluble proteins, increased the accumulation of chlorophylls and water-soluble carbohydrates. As a result of epibrassinolide action the activity of peroxidase and ascorbateoxidase in cells was higher that was conductive to decreasing the concentration of activated forms of oxygen and delaying the oxidizing disintegration of their components. In more sensitive genotypes with Roland's nucleus the positive influence of epibrassinolide on seedling growth in presence of toxic fungicide was also caused by the recovery of structural protein content and the activation of initial photosynthetic reactions related to water photooxidation.

Epibrassinolide did not affect the biological efficiency of fungicide 'Vintsit' against root rot but protected the plants against agents of spot diseases.

As a result of combined application of fungicide 'Vintsit' and phytohormone epibrassinolide the mass of 1000 grains and mass of grain from one plant increased in comparison with the application of only fungicide especially in more sensitive isoplasmatic genotypes with Roland's nucleus.

The getting results are useful for better understanding how brassinosteroids involved in mechanisms of plant adaptation and may serve as the theoretical basis for agricultural application of brassinosteroids in complex with fungicides for seed treatment.

COMPOSITE PROTECTIVE SUBSTANCES OF NATURAL ORIGIN

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Natural mixtures of growth-regulating compounds interacting during growth and metabolism processes are the basis of composite protective substances. Phytohormone epibrassinolid and phenolcarboxylic acids can be attributed to such compounds. In terms of a physiological sense, both groups of substances have much in common. They are able to regulate growth processes changing metabolism behavior, to interact with other phytohormones and to affect formation of defense reactions. However, their interaction in the above processes was not studied and possible effects were not revealed.

Both initial components and mixtures of the phytohormone with ferulic and salicylic acids in 10^{-6} M concentrations did not affect stalk growth of spring wheat but increased appreciably the time of leaf functioning that indicated metabolism rate. Mixtures of epibrassinolid with phenolcarboxylic acids exerted a maximum effect on leaf color.

All phytoregulators and their mixtures increased the content of green pigments and carotenoids in wheat plants, individual substances increasing to a greater extent at the initial stage after treatment (shooting stage) and mixtures of epibrassinolid with phenolcarboxylic acids doing at the final stage (milky ripeness). Prolonged preservation of green color in wheat leaves can be accounted for by this fact.

Epibrassinolid, phenolcarboxylic acids and their mixtures reduced formation of lipid peroxidation products, particularly sharply at the end of vegetation (milky ripeness). Thus, ferulic and salicylic acids decreased their accumulation almost twice, mixtures did by 32% and the phytohormone reduced by 23%, therefore, all compounds and their mixtures acted as antioxidants.

The yield of water-soluble substances from wheat leaves at the shooting stage increased twice under the phytohormone effect, by 50% under the effect of phenolcarboxylic acids and to a minimum, extent under mixture treatment. An increase in the yield of water-soluble substances from wheat leaves is related to phytosynthesis activation and damage of wheat leaves and stalks by phytopathogenic fungi which reached 60% at this period. Metabolism rearrangement under the effect of the natural phytoregulator mixture was concluded to favor plant protection.

SESSION 4:
Lipid signaling

ROLE OF PHOSPHOLIPASE C IN ABA SIGNAL TRANSDUCTION

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Phospholipase C (PI-PLC; EC 3.1.4.3) is a crucial lipid-associated enzyme that hydrolyzes membrane bound phosphatidylinositol- 4,5-bisphosphate (PIP2) to produce inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. These two second messengers play key roles in amplifying extracellular signals and regulating of various intracellular processes triggered by abiotic and biotic stimuli. However the contribution of the phosphoinositide signaling pathway in plant acquisition of stress acclimation is not well understood at present.

One of the initial components of adaptation strategy is activation of plant protective mechanisms by ABA. Some reports point to the role of phosphoinositide-specific phospholipase C (PI-PLC) in ABA signal transduction. We focus our research is on the role of PI-PLC in ABA signaling in Zea mays L and *Pisum sativum L.* plant. The effect of ABA on the changes in level of polyphosphatidylinositols PI(4)P, PI(4,5)P2 and Ins(1,4,5)P3 of maize leaves were investigated. Ins(1,4,5)P3 levels have sharply increased within of ABA treatment in compared with control. Changes in Ins(1,4,5)P3 were associated with decrease in PI(4)P and PI(4,5)P2 levels. Our data indicate that the transient Ins(1,4,5)P3 production occurs on early stage of the ABA action as a result of PI-PLC activation.

One of the most sensible systems that activated after ABA perception is guard cells movement implementation. Application of ABA to stomata results in a decrease of the stomata pore that is achieved by inhibiting the processes associated with stomata opening and promoting the cellular events that occur during stomata closure. Neomycin sulfate, a well-known PIP2-PLC inhibitor, was used to examine the effects of PI-PLC and abscisic acid on stomata aperture in epidermal strips of pea leaves. Taken together, our data suggest that phospholipase C mediates the ABA effects on stomata aperture.

This study was supported by the grants of Fundamental Researches State Fund of Ukraine *F10/24-2005, F14/247-2007.*

PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLINOSITOL ARE ALLOSTERIC REGULATORS OF 5-LIPOXYGENASE FROM POTATO TUBER

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Lipoxygenases are a class of non-heme iron dioxygenases that catalyze the incorporation of oxygen into 1,4-*cis,cis*-pentadiene containing fatty acids to form hydroperoxides. This reaction is a key step in the synthesis of signaling molecules such as jasmonic acid and related compounds, antimicrobial and antifungal compounds and a plant-specific blend of volatiles. Lipoxygenase products in plants are implicated in regulation of growth, senescence, and stress-related response. Under typical physiological pH, the fatty acids are located in membrane structures of the cell and therefore enzyme translocation to the membrane surface and interaction with its components, such as phospholipids (PL), will be an important for lipoxygenase activity regulation. Early was showed that phosphatidylcholine (PC) or phosphatidylinositol (PI) (0,005 – 0,3 mM) decrease or increase the steady-state velocity of linoleic acid ($C_{18:2}$) oxidation by 5-lipoxygenase (5-LO) from potato tubers, respectively.

The mechanism of interaction between enzyme and widespread phospholipids was studied in the present work. 5-LO was extracted from *Solanum tuberosum* tubers cv Lugovskay and purified by a 25-50% ammonium sulfate precipitation, dialysis, ion exchange chromatography (DEAE-cellulose pH 7,5), and hydrophobic chromatography (Butyl-sepharose pH 7,5). 5-LO shows positive cooperatively of linoleic acid ($C_{18:2}$) binding with a Hill coefficient of 4±0,22 in micelle system (0,02% Lubrol PX). PC and PI (0.1 mM) reduce a Hill coefficient under 1,196±0,14 and 1,67±0,13 respectively. It was demonstrated that PC decrease the maximum velocity of linoleic acid oxidation by 5-LO while PI increase this value.

The obtained findings indicate to the mechanism of allosteric regulation of the 5-LO activity by PC and PI. The effect of PL on the maximum velocity of reaction depends on molecule charge. Probably the additional negative charge is essential for proton dependent stage of lipoxygenase catalyze. This is agreement with the activatory effect of acidic amphiphiles on the 5-LO from potato.

Evidently that the role of the membrane lipids composition in lipoxygenase catalyze consist in both binding protein to membrane surface and regulation of enzyme activity.

NITROGEN OXIDE AND WOUNDING INFLUENCE ON THE ACTIVITIES OF PHOSPHOLIPASES D AND A_2 IN TOMATO LEAVES

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The great interest in research of plant NO-synthase signalling system is connected with a special role of nitrogen monoxide (NO) to have a wide spectrum of biological activities. Basing on literary data, it is possible to suppose, that phospholipid and NO-dependent pathways cooperate in defensive reactions of a plant organism in response to pathogen action and influence of pathotoxins and elicitors.

The aim of the present work was to study the influence of NO-donor sodium nitroprusside (SNP) and its antagonist methylene-blue (MB) on activity of phospolipases D (PLD) and A_2 (PLA_2) and also to reveal the possible interrelation between NO as a signal molecule and activities of PLD and PLA_2.

These studies were performed on leaves of tomato plants (variety "Vilina", resistant to *Phytophthora infestans*). The leaves were placed in water solutions of SNP (100 μmol), methylene-blue (10; 1; 0,1; 0,01; 0,001 mmol) and distilled water (control) for 30 min right after cutting and then were homogenized. Measurement of PLD and PLA_2 activity *in vitro* was carried out by spectrofluorometric method using fluorescent-labeled phosphatidylcholine analogue.

It was established, that wounding caused by cutting resulted in an increase of PLD and PLA_2 activities in comparison with the control already in 30 min after exposure (by 1,2 and 1,4 times, accordingly). Incubation of tomato leaves in the presence of 100 μmol SNP (without MB) results in significant increase of PLD activity commensurable with the effect caused by wounding.

At high content of MB (1-10 mmol) the effect of increase of PLD activity caused by wounding was completely inhibited and PLA_2 activity was considerably reduced.

But at more low content of MB (1-100 μmol) inhibition of phospholipase activities was inefficient.

PLD and PLA_2 activities in wounded tomato leaves remained to be high in the presence of 100 μmol SNP and various concentrations of MB as well as in absence of the mentioned compounds, and same, as in the presence of only one SNP (100 μmol). Inhibitory action of MB (all concentrations) was removed in the presence of 100 μmol SNP, and values of PLD and PLA_2 activity were commensurable with the effect caused by wounding.

The obtained data testify to close interrelation between NO-synthase and phospholipid metabolic pathways in plants.

The cross-talk between NO, phospholipid and phytohormone regulatory pathways of plant cell is discussed.

CHANGES OF UNSATURATION OF FATTY ACIDS OF SUGAR BEET CALLUS TISSUES LIPIDS AT BACTERIAL STRESS

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Adaptable properties of plants are caused by features of structure and a metabolism of lipid components of membranes. Stability of membranes connect with qualitative and quantitative changes in phospholipids and fatty acids composition. The increase of unsaturated fatty acids promotes increase of plants resistance to bacterial stress. It explain more friable packing of unsaturated fatty acids, than saturated, in bilayer and areas of contact phospholipids with proteins, that gives to a membrane the big plasticity, fluidity, flexibility.

The aim of our work is studying of bacterial stress impact, which induced *Pseudomonas wieringae,* on changes of unsaturation of fatty acid of sugar beet callus tissue 'Roberta'.

The object of research was callus tissue of sugar beet of hybrids 'Roberta'. We used Murasige-Scoog medium which contains the *P. wieringae* lifeless cells in concentration 0,4ml cell suspension/50ml medium and 0,5ml cell suspension/50ml medium for callus cells of sugar beet cultivation. *P. wieringae* lifeless cells was obtained by heating at temperature 100ºC in 2 hour. Callus cells lipids were extracted by Bligh and Dyer Method. Fatty acid methyl esters were prepared and identified by gas chromatograph Agilent 6890N with mass-spectrometer detector 5973 inert (Agilent, Waldbronn, Germany).

Lipids of callus of triploid hybrids Roberta have been characterized by presence of fatty acids with number of carbon atoms from C_{15} to C_{24}. In structure of callus tissues lipids the prevailing saturated acid was hexadecanoic acid – 18,89 %. Among nonsaturated acids prevailed 9,12-octadecadienoic acid – 23,20 %, cis-octadecenoic acid – 45,4 %. The contents of other fatty acids did not exceed 3 %.

Addition of *P. wieringae* lifeless cells in a nutrient medium caused change of the contents of fatty acids in callus tissues lipids of hybrids Roberta, is especial 9,12-octadecadienoic acids. At concentration of *P. wieringae* lifeless cells 0,4ml cell suspension/50ml medium the contents 9,12-octadecadienoic acids has been increased to 30,6 %, whereas cis-octadecenoic acids has been decreased to 30,7 % in comparison with the control. If concentration of *P. wieringae* lifeless cells were 0,5ml cell suspension/ 50ml medium, the contents of 9,12-octadecadienoic acids has been increased to 36,1 %, cis-octadecenoic acids has been decreased to 27,1 %. The increase in a ratio 9,12-octadecadienoic acids / cis-octadecenoic acids probably indicates to activation of work desaturatical systems or synthesis 9,12-octadecadienoic acids de novo.

Thus, adaptation of callus tissue of sugar beet triploid hybrids Roberta to *Pseudomonas wieringae* is connected to increase in nonsaturation lipids, mainly for the account 9,12-octadecadienoic acids.

CHANGES OF LIPOXYGENASE AND HYDROPEROXIDE LYASE ACTIVITIES DURING STORAGE AND GERMINATION OF POTATO TUBERS (*SOLANUM TUBEROSUM L.*)

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Most phyto-oxylipins which constitutes a large class of diverse oxygenated polyunsaturated fatty acids and their derivatives are produced through the lipoxygenase pathway.

The oxygenation of polyunsaturated fatty acids by lipoxygenases (LOX) represents the first step in fatty acid metabolites synthesis. Hydroperoxide lyase (HPL) is a part of the lipoxygenase pathway that catalyses the conversion of fatty acid hydroperoxides into aldehydes and oxo-acids. Products of the lipoxygenase pathway play an important role as growth and senescence regulators, antimicrobial compounds and signal molecules.

Changes of both lipoxygenase and hydroperoxide lyase activity have been monitored during storage and germination of potato tubers (*Solanum tuberosum L.*).

LOX and HPL activity gradually increased beginning from apical dominance stage, reaching maximum at multiple sprout stage with a decline at later stages of development.

The maximum activity of both enzymes in bulbs occurs in multiple sprout stage. LOX activity increased more than two-fold in comparison with their activity in apical dominance stage, where HPL activity was not detected. In daughter tuber stage LOX and HPL activities decrease two- and three-fold, respectively. HPL activity was also synchronously enhanced with LOX activity in multiple sprout stage.

Moreover, LOX and HPL activities in seedlings and in bulbs were different. HPL activity in seedlings was more than two-fold higher in comparison with bulbs and vice versa for LOX activity.

These observations suggest that lipoxygenase pathway enzymes (lipoxygenase and hydroperoxide lyase) and their products play a substantial role during germination of potato tubers.

CHILLING TOLERANCE DIFFERENCES OF VARIED POTATO CULTIVARS AND ITS GENOTYPES ACQUIRED THROUGH TRANSFORMATION

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As a part of chilling tolerance mechanisms research were determined two proving pathways – saturation index changes and accumulation of protective material (e.g. sugars). Effectiveness and impact of changes in these parameters in conjunction to relative chilling tolerance is discussed.

Our study was carried out with potato plants (*Solanum tuberosum* L.) cultivars Désirée and Desnitsa. Plants of Désirée cv. were transformed with vector carrying yeast invertase gene under the control of tuber-specific patatin promoter B33 class I, fused with proteinase II inhibitor leader peptide to provide enzyme location in apoplast. Plants were obtained in cooperative work of Max Plank Institute of Molecular Plant Physiolgy (Golm, Germany) and Laboratory of Growth and Development, Timiryazev Institute of Plant Physiology RAS, and gently provided by employees of the last. Plants of Desnitsa cv. were transformed with vector carrying dasA gene, encoding Δ12-acyl-lipid desaturase of fatty acids and thermostable lichenase reporter gene (licBM3). Both genes were placed under the control of constitutve 35S CaMV promoter. Agrobacterial transformation was performed according to method of micro-tuber transformation developed in Departnent of cell biology and biotechnology, Timiryazev Institute of Plant Physiology RAS. Both cultivars were grown in vitro at 22^{o}C under diffused fluorescent light (16 hours a day, LB-80 lamps, 4 klx) on MS nutrient medium, containing 2% of sucrose. Chilling was performed by exposition of plants at -7^{o}C during 30 min.

Potato plants of medium-speed maturating cv. Désirée is a product of Dutch selection, while early-to-medium maturating cv. Desnitsa plants appeared at Bryansk experimental station, so a priori we could suppose they would differ in chilling tolerance between cultivars and also between transgenic and wild genotypes of the same cultivar.

Comparison of the relative chilling tolerance of studied genotypes based on electrolyte leakage and lipid peroxidation intensity. As a result of experiments carried in our laboratory electrolyte leakage differences were shown for nontransformed plants of studied cultivars. Thus, control plants of Désirée cv. comparing with Desnitsa cv. demonstrated lower membrane permeability index that might mean higher tolerance.

Experiments with transgenic potato plant leaves either of Desnitsa or Désirée cv. revealed higher tolerance of their genotypes in comparison to their controls. Transformed plants possessed membranes that were more stable at low temperature than control ones. We suppose that described differences in membrane stability are corresponding to high concentration of soluble carbohydrates in case of Désirée cv. and low saturation index in case of Desnitsa cv., resulting in more effective signal and protective systems.

The study is supported by Russian Foundation for Basic Research (project no. 04-04-48476).

INFLUENCE OF PLANT HORMONES AND GROWTH REGULATORS ON 9-LOX ACTIVITY OF POTATO MINITUBERS (SOLANUM TUBEROSUM L.)

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Plant lipoxygenases (LOXs) are a functionally diverse class of dioxygenases implicated in physiological processes such as growth, senescence, and stress-related responses. LOXs incorporate oxygen into their fatty acid substrates, and they produce hydroperoxide fatty acids that are precursors of jasmonic acid and related compounds.

Tuber formation in potatoes (Solanum tuberosum L.) is a complex developmental process that requires the interaction of environmental, biochemical, and genetic factors. Several important biological processes like carbon partitioning, signal transduction, and meristem determination are involved (Ewing and Struik, 1992). Tuber development at the stolon tip is comprised of biochemical and morphological processes. Both are controlled by differential gene expression (Hannapel, 1991; Bachem et al., 1996; Macleod et al., 1999) with most of the work focusing on the biochemical processes, including starch synthesis (Abel et al., 1996; Preiss, 1996; Geigenberger et al., 1998) and storage protein accumulation (Mignery et al., 1984; Hendriks et al., 1991; Suh et al., 1991). Much less is known about the morphological controls of tuberization, although it is clear that phytohormones play a prominent role (Koda et al., 1991; Xu et al., 1998, Sergeeva et al., 2000). Despite considerable work on the physiology of tuber development, the molecular mechanisms that control the changes in cell growth during tuberization have not been identified.

Potato LOXs are encoded by a large multigene family. Several LOX cDNAs have been isolated from potato tubers, roots, and leaves (Geerts et al., 1994; Casey, 1995; Kolomiets et al., 1996a; 1996b; Royo et al., 1996; Fidantsef and Bostock, 1998). LOX expression has been detected in developing tubers, and several groups have proposed that LOXs are involved in potato tuber growth (Bachem et al., 1996; Kolomiets et al., 1996a; Royo et al., 1996), but until now, there were no reports that demonstrated this involvement. Although LOXs are known to function in diverse physiological processes, this study is the first definitive proof of LOX involvement in the regulation of tuber development.

Several hormones, i.e., gibberellin, homo-brassinosteroid and growth regulator – salicylic acid, reportedly play a role in tuberization. The potato tuber discs were incubated with GA (10 mg/l), homo-brassinosteroid (10^{-7} M) and salicylic acid (10^{-7} M) during three hours. Proteins were extracted from minitubers as describes by Grimes et al. (1993). LOX activity was determined by Shimizy et al. (1990). Spectrophotometric measurements of the increase in A_{234}, caused by the formation of conjugated diene structures.

Our data witness that plant hormones (gibberellin, homo-brassinosteroid) and growth regulator (salicylic acid) brings about the change of the activities LOX. It was found that GA brought about an increase LOX activity (2.40 μmol sec^{-1} g^{-1} protein) with linoleic acid as substrates. Herewith exists the protein expression under action GA. Homo –brassinosteroid influenced on LOX activity (2.04 μmol sec^{-1} g^{-1} protein) relatively control variant (0.47 μmol sec^{-1} g^{-1} protein), but did not manifest themselves on the protein expression. SA weakly actuates LOX (0.70 μmol sec^{-1} g^{-1} protein), however powerfully expressed of the proteins, occurs the appearance an integer spectrum new proteins.

Overall, these results suggest that the expression of the tuber LOX genes is important in controlling tuber development.

NEW IN HORMONAL REGULATION OF α-AMYLASE IN GERMINATING WHEAT GRAIN

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One of the hot points of modern biology is the investigation of hormonal regulation of processing and activation of the enzymes. In this reason it is very interesting to investigate hormonal regulation of α-amylase in germinating wheat seeds. This enzyme plays key role in catabolism of starch – the basic energetic source for development of plant during its heterotrophic nutrition.

It is well known that the main hormone in regulation of α-amylase is the gibberellic acid (GA). It was supposed that this enzyme switches on the biosynthesis of α-amylase. However as it was shown by us almost all molecules of α-amylase are in latent state in the cells of aleurone layer of wheat seeds. And *de novo* synthesis of α-amylase is very low. Thus, a question arise on the other mechanism of GA action.

We suggested, that GA induces the phospholipase A in germinating wheat seeds. The phospholipase A converts phospholipids into their lisoforms. Lysophospholipids have the properties of strong detergents. They very easily solubilise the cell membranes.

We tested the effect of the lysophospholipids which was formed by GA and strong artificial detergent triton X-100. Both detergents have the same effects. They convert latent amylase to active state. And on the electrophoregramme appear 4-5 new bands of α-amylase.

It is well known, that during germination the molecules of protein undergoing glycosilation process in Golgi apparatus. Tunicamycine strongly inhibits this process. We tested the effect of tunicamycine on germinating wheat seeds and showed that the glycosilated α-amylase bands did not appeared in this case.

Now the interest of scientists sharply increasing to a new powerful bioregulator - nitrogen oxide (NO). We tested effect of NO on germinating wheat seeds and showed that NO causes the essential changing in electrophoretic spectra of α-amylase but the total activity of amylase not changed. It speaks that NO causes the modification of charging of molecules of α-amylase.

We also investigated very interesting effects of action of abscisic acid (AbA) on α-amylase in germinating wheat seeds. So, it was established, that under action of AbA there was a sharp decrease of α-amylase activity during germination. This speaks that AbA cancels the effect of GA during wheat seeds germination.

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.

SESSION 6:
Plant stress signaling and response

MODULATING INFLUENCE OF GIBBERELLIC ACID IN HYPERTHERMIA IN WHEAT GRAINS

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The speed of reserve matter's splitting in seeds depends on external and internal conditions. We investigated the impact of exogenous gibberellic acid - GA ($1 \cdot 10^{-5}$ M) on proteolysis and resistance of wheat seedling under high temperature stress.

Hyperthermia lead to decrease of proteolysis level (pH 3.5) in wheat grains and similar but not statistically authentic changes in above-ground parts of wheat. Negative influence of high temperature on proteolysis is released partially through GA during 2-days grains.

GA + hyperthermia in comparison with hyperthermia showed changes in thermoresistant grains that were grown in water: electroconductivity, pH near roots and O_2 in water as well.

Also we showed that electroconductivity in solution dependents on plasmatic membrane permeability of root system and that grows under high temperature changed less with GA. GA decreased acidulation of extracellular solution by root fibrils, especially at 44^oC. In case with GA at this temperature we found high level of soluble O_2 in the solution near to roots. It can be dew to normalization of the inspiration that was revealed under hyperthermia.

Thus we showed the role of GA in response of high temperature stress on wheat grains. GA reduces intensity of negative stress on plants.

EFFECTS OF ABSCISIC ACID ON ACTIVITY OF AMIDASE, CYSTEINE PROTEASES AND TRYPSIN`S INHYBITORS OF WHEAT SEEDLINGS IN COLD HARDENING

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The aim of this work was to study effect of exogenous abscisic acid (ABA) on activity of amidase, cysteine proteases and trypsin`s inhibitors of wheat seedlings in initial period of cold hardening.

Winter wheat (*Triticum aestivum L.*, cv. Moskovskaya 39) seedlings were grown inside of rolls of filter paper moistened with Knop nutrient solution in a growth cabinet under constant conditions. One-week-old seedlings were exposed to hardening temperature 5˚C during two days. ABA was added to a nutritious solution for one day prior to the beginning of hardening. Cold tolerance was judged from the temperature causing the death of 50% of palisade cells in leaf discs after 5-min frozen in a microrefrigerator. Amidase activity was assayed with synthetic substrate – BAPA (Nα-Benzoyl-DL-arginine-4-nitroanilid-nydrochlorid), activity of cysteine proteases – on the modified method Kunitz, activity of inhibitors – on suppression of enzyme's activity.

Our studies showed that exogenous ABA enhanced the resistance in unhardened wheat seedlings and promoted plant cold resistance.

It is necessary to note, that through 24 h after processing plants by a solution ABA the activity of cysteine proteases was in 2 times less, than at control (not processed ABA). The activity of amidase and trypsin`s inhibitors exceeded those at the control in 2-3 times.

In process of cold hardening of the plants, which have undergone by preliminary processing ABA, the activity of amidase and cysteine proteases did not vary practically. The activity of trypsin's inhibitors increased a little bit through 30 min, and further it was reduced gradually during the next two days.

Thus, our data allows us to conclude that in the process of cold hardening and increasing of initial cold-resistance's rate of plants were processed ABA (without hardening) amidases participate in updating and elimination of proteins which not carrying out necessary functions, and also provide a crate monomeric substrates for synthesis the proteins *de novo*, which participate in formation of cold-resistance. We also consider that trypsin's inhibitors represent as regulators of protease activity and prevent premature degradation of synthesized proteins, thus supporting process of formation of the increased cold-resistance.

IAA IN ORGANS OF *PERSICARIA AMPHIBIA (L.) DELARBRE* IN VARIOUS CONDITIONS OF GROWING

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The reaction of plants on the changes of the environmental factors mediated by phytohormones. Although numerous cases of studies on quantitative changes in IAA and its role played in actions of this or that stressor are described in the literature, the results of these studies remain rather contradictory. Nowadays the hormonal control of growth and development of plants that are able to grow under natural adverse conditions for some time remains almost unexplored. Therefore, our studies were aimed at the investigation of quantitative changes in IAA of *Persicaria amphibia* (L.) Delarbre – plant species that are characterized by high degree of adaptation to the water-level fluctuation.

The studies showed that the quantitative content of IAA in organs *P. amphibia* varied depending on growing conditions and stage of ontogenesis. Younger organs, in comparison with older ones were characterized by mostly by a higher content of IAA. During ontogenesis a water form and leaves of ground forms one showed some increase in free IAA content as result of redistribution between free and conjugated forms while in internodes of ground forms there were observed some decrease in the content of both forms of the hormone. Water deficit, occurring in the beginning of vegetation, resulted in some increase both in free and conjugated IAA content in the upper part of shoot. An increase of the IAA content in the beginning of drought effect appears to be associated with an increase in the level of tryptophane which is a precursor of the synthesis of IAA, with its function of breathing activation, with the maintenance of growth renewal and with other physiological processes in reparation period. During flowering the water deficit also caused some increase in IAA content in upper leaves. In upper internodes of the main and lateral shoots of ground forms an increase in free IAA content was insignificant while in lower internodes its quantity was similar in plants growing different conditions. Generative organs of ground forms were characterized by an insignificant content of IAA.

Thus, the above mentioned results of the researches allow to assume the participation of IAA in adaptive reactions of *P. amphibia*.

OVEREXPRESSION OF AN ARABIDOPSIS CYTOCHROME P450 GENE INCREASES DROUGHT STRESS RESISTANCE

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Plant cytochrome P450 genes have been known to be implicated in the biosynthesis of diverse secondary metabolites including plant hormones. Transgenic *Arabidopsis* overexpressing an *Arabidopsis* cytochrome P450 showed the enhancement in drought stress resistance. To gain molecular features of the increased drought stress resistance in the transgenic *Arabidopsis*, we performed whole genome microarray analysis. Microarray analysis revealed that expression of seed storage and ABA-responsive or drought/cold stress-related genes were up-regulated in the transgenic *Arabidopsis*. Microarray data was further confirmed by RT-PCR. Seed size and weight were also increased in the transgenic lines compared to wild-type. SDS-PAGE analysis showed that seed storage proteins such cruciferin and 2S albumin were highly accumulated in the transgenic seeds. Germination rate of transgenic seeds was greatly lower and more sensitive to low concentration of ABA than wild-type. The lower germination rate of transgenic seeds was recovered by an ABA biosynthesis inhibitor, fluridone. This data support that overexpression of the cytochrome P450 may increase endogenous ABA level. To develop rice transformants coping with drought stress, we introduced the P450 gene into rice. We found eight homologous genes for the Arabidopsis P450 gene from rice genome database. We analyzed their tissue expression pattern and responsiveness to drought stress and exogenous ABA treatment. To elucidate the biological function of the rice homologous genes, we are also generating transgenic *Arabidopsis* and rice plants overexpressing the rice homologs. This work was supported by a grant (Code 20070301034028) from the BioGreen21 Program, Rural Development Administration, Republic of Korea.

PARTICIPATION OF SALICYLIC ACID AND LECTIN IN THE GROWTH AND ADAPTIVE PROCESSES OF CEREALS DURING THE INFECTION BY FUSARIOSE

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The whole raw of compounds, participating in the transmission of signals regulative immunity and growth of plants was found. The salicylic acid and lectin can belong to these compounds. The study of the influence of exogenous salicylic acid and lectin on the plant growth processes and activity of endogenous lectins, phehylalanineammonia-lyase and trypsin inhibitor activity of the seedlings of the winter and spring barley during the infection by fusariose, was the purpose of the present research.

The six-dayseedlings of winter wheat (Triticum aestivum L.) and spring barley (Hordeum vulgare L.) genotypes differentiating by their resistance towards the fusariose, were used in the researches. 2 mM salicylic acid was used in the experiments. As the source of the infection, the suspensions of pathogens Fusarium graminearum and Fusarium culmorum in the concentration of the 10 million conidia/ml were taken. Lectin was picked out from the Soya seeds by the method of ethanol fractionating of Ryagas and Osgudi. Lectin was used in concentration of 50 and 100 µg/ml. Lectin activity was determined by their ability to agglutinate the trypsin red corpuscles of white rats at the room temperature by the method of Lucik. The activity of the trypsin inhibitor was determined with the help of synthetic substrate N-benzoyl-arginine-4-nitroanilide. The activity of the phenilalanineammonia-lyase was determined by the method of Zucker, modified by us.

It was established that the growth of the seeds in the environment, containing the salicylic acid or lectin, positively influenced on the growth processes of uninfected and infected plants. The character of the influence of salicylic acid and lectin depended on its concentration. It was shown that the changes of the phenylalanineammonia-lyase, trypsin inhibitor and lectin activity, happened under the influence of the salicylic acid, lectin and fusariose infection, depend on the level of the resistance of genotypes towards the fusariose, plant organ, genus of culture and influencing factor. A difficult interaction, directing on the stimulation of the growth processes and saving or increasing of plant resistance towards the present pathogen, was the result of the joint action of the salicylic acid or lectin with the fusariose infection. The received results allow us to suppose the participation of the lectin and salicylic acid in adjusting of the different ways of metabolism, in the propitious direction for the growth and development of plants and the activation of the biochemical system of protection. Further researches may allow us to use the lectin and salicylic acid preparations at the development of the new methods of protection of cereals from the infection of the fusariose mushrooms, based on the activation of the natural protection mechanisms of plants.

PIGMENT COMPOSITION IN CYANOBACTERIAL MUTANTS AT OXIDATIVE STRESS INDUCED BY PARAQUAT

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The effects of herbicide paraquat on the pigment composition of the cyanobacterium *Synechococcus* sp. PCC 7942 and its *sodB*$^-$ mutant, and the cyanobacterium *Synechocystis* sp. PCC 6803 and its *katG*$^-$ mutant were examined in comparison with the effects of higher light conditions. Pigment composition of the *katG*$^-$ mutant of *Synechocystis* sp. PCC 6803 is just similar to the wild type strain. The *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 is characterized by lower chlorophyll *a* content but higher chlorophyll/phycocyanin ratio as compared with the wild type.

The both mutant cultures grew slower than their wild type strains at higher light conditions (300 μmol photons m^{-2} s^{-1}) but did not behind from them at irradiance of 30 μmol photons m^{-2} s^{-1}. When moved to strong light, total pigments decreased in the all strains and the ratio of chlorophyll to phycocyanin increased. The *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 showed a higher ratio of chlorophyll to phycocyanin under all conditions.

The paraquat, methyl viologen (MV), in concentration 10 μM caused to dieing of every investigated strain. The each culture stopped to grow and became colorless after 8 hr of incubation with the presence of 10 μM MV. The in concentration of 0.5 μM had no significant negative effect on growth and pigment content of the wild type strain of *Synechococcus* sp. PCC 7942 and the wild type strain and *katG*$^-$ mutant of *Synechocystis* sp. PCC 6803. At the same time 0.5 μM MV suppressed growth of the *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942, although this mutant did not die and did not bleach at such treatment; the pigment composition remained unchanged. We suppose that activation of catalase [1] allow survival of the cyanobacterial mutant lacking cytosolic superoxide dismutase under oxidative stress induced by MV.

Thus, the changes in pigment composition of the cyanobacterial cell, such as decreased chlorophyll *a* content and increased ratio of chlorophyll to phycocyanin under the higher light conditions, may be considered as adaptive reactions which are not related to *katG* or *sodB* genes. On the other hand, defense of cyanobacterial cell against the stress induced by MV, is related to antioxidant enzymes but not to changing pigment composition. The obtained results are in agreement with assumption about different primary targets of oxidative stress induced with different inductors [2-5].

The present work was supported by grant from the U.S. Civilian Research and Development Foundation; project # RB1-2351-PU-02.

References

1. Fomina I.R. *et al*. //Proceedings of the 6th International Symposium "New and Nontraditional Plants and Prospects of Their Utilization", Moscow, 2005, 1: 413-415.
2. Herbert S.K. *et al*. //Proc. Natl. Acad. Sci. USA, 1992, 89: 8716-20.
3. Thomas D.J. *et al*. //Plant Physiol., 1998, 116: 1592-1602.
4. Thomas D.J. *et al*. //Plant Physiol., 1999, 120: 275-282.
5. Avenson T. *et al*. // Plant Cell Physiology, 2007, submitted.

RELATIVE ESTIMATION OF THE MODIFICATION IN THE IAA LEVEL UNDER THE HIGH TEMPERATURE INFLUENCE ON THE *TRITICUM AESTIVUM* L. AND *AZOTOBACTER CHROOCOCCUM*.

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The plant hormones controlling growing and morphogenesis certainly play the important role in the realization of the defence program too. It remains unclear what stage (alerts or adaptation) of the stress reaction development the indol-3-acetic acid is associated. Our experiments included revealing the kinetics of the early modifications in the indol-3-acetic acid quantity after the heat shock influence on wheat seedlings (*Triticum aestivum* L.) and bacteria (*Az chroococcum*). The target of these studies was to reveal the IAA participation in the initial stages of the stress reaction by organisms with different extent of evolution development. Our special interest was in connection with IAA hyperproduction by the procaryotes. The endogenous IAA level in bacteria was modulated by adding the tryptophan in concentrations 20 - 200 mg/ml, the control was without the IAA precursor in the cultural solution. The objects of this research were green and etiolated wheat plants considered to the fact of IAA metabolism photosensivity. The heat shock was modeled by the exposing tubes with the bacterial suspension or plants for 5-120 minutes (temperature +42-47 ^{0}C). The IAA in the cultural solution was measured using the immunoenzyme assay or by the reaction with the Salkovsky reagent (colorimetric analysis). The estimation assay of free and conjugated IAA pools was studed by the immunoenzyme assay after the alkaline hydrolysis in shoots and roots of wheat seedlings. It is revealed that the reaction of the hormonal system in the response to the heat shock developed early and in the 5-15 minutes after the hyperthermical action the temporary efflux of free IAA was fixed in the shoots and roots of green wheat plants. The differences between green and etiolated plants due only to the value of the efflux amplitude. The maxima of the free IAA accumulation was in 5.5-15.0 times more in green and in 2.3-3.0 in the etiolated seedlings then the level in untreated control plants. The founded differences in the hormonal response of heated plants can be due to the two mechanisms: 1.the balance breakage in the system “free/ conjugated IAA” (in the etiolated plants in comparison with the lighted seedlings the speed of IAA conjugating is lower); 2. the changes in the enzymatic system “IAA oxidase/its inhibitor”(etiolated seedlings are characterized by elevated enzyme activity and the lower inhibitor contence).The first control mechanism is responsible in the growth of free IAA (active form) concentration in the stress conditions. The second mechanism defined the temporality of the IAA efflux, deelevating the increased hormonal concentration. In bacteria every decrease of growth value (such as after the heat shock action, nutrition deficit or anaerobiosis) induced the IAA hyperproduction. The analogical reaction is characterized the substances of the second metabolism which functioned as detoxicants of the first metabolism substances. Our experiments have shown that tryptophan is specifically toxic component for the procaryotic organisms (probably that connected with the vacuole absence in them). Thus, we can suppose the IAA biosynthesis activation in this case can be the activation of the tryptophan breakage speed (detoxication rate). Furthermore, the IAA been the weak acid can act in the mechanisms of the pH-homeostasis. The last was founded in the dynamics of pH_{in} comparison in stressed by the high temperature action and untreated *Az. chroococcum* in the model with the adding and the absence of the IAA precursor in the cultural solution. In conclusion, the general response reaction - the temporary heat-induced growth of IAA level in the outcellular space (procaryotic organisms) and in the cellular and/or apoplastic sites (eucaryotes) has been found. Both studying objects indicated that concentrational efflux of IAA take place with the growth depression what confirm its signal function. In the early stages of evolution both tryptophan breakage activation and intracellular aciding in the stress conditions played a great role in the signal forming. Later the complexity of the controlling system included enzymatic oxidation reactions and IAA conjugates forming has occurred.

THE EFFECTS OF OSMOTIC STRESS ON PHOSPHOLIPASES D ACTIVITY OF MAIZE CELLS AND TRANSGENIC TOBACCO PLANTS CELLS EXPRESSION OF *CAX*1

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Comprehension of the mechanisms by which plants perceive the state of environmental conditions and transmit signals to cellular metabolism for adaptive responses activation is a key fundamental question in biology. Various signalling cascades, associated with abiotic stress responses, are triggered by the activation of phospholipases C and D enzymes that are responsible for production of lipid second messengers. Most of phospholipases D isoformes require Ca^{2+} for their action, but exactly how Ca^{2+} affects PLD activity is not well understood for now. Ca^{2+} as the second messenger is also involved in ROS-cleaving enzymes activation cascades and other adaptation processes in plants. Maize cultivar Goverla and Tobacco cultivar KY160 overexpressing *cax*1 (Calcium Exchanger 1), which is unable to conduct rapid efflux of Ca^{2+} from tonoplast, were used in the study. PLD activity was monitored as the production of phosphatidylbutanol (PBut) *in vivo*. ^{33}P-pre-labelled plant tissues were treated with manitol, or buffer (control) for 5 - 30 min, then incubations were stopped, the lipids were extracted and separated by ethyl acetate TLC. Our results demonstrated that moderate osmotic stress induce sharp accumulation of ^{33}PBut in tissues of maize and tobacco plants, both transgenic and wild type, at the early stages of osmotic stress treatment that indicates the role of PLD in osmotic stress signal perception. We have also shown that transgenic tobacco plants cells overexpressing *cax*1 were poorly acclimated during one week under moderate osmotic stress conditions which confirms that $Ca2^{+}$ is involved in cell adaptive responses development. The plant adaptation level was quantitatively assayed by the measurement of MDA (malonic dialdehyde) accumulation, as a marker substance of irreversible cell membranes damage, and assaying the activity of ROS-cleaving enzymes (catalase, peroxidase, superoxide dismutase) in plant tissues. Treatment with PLD activity inhibitors such as Neomycin and 1-Butanol resulted in decrease of adaptation capability of tobacco plants, which is consistent with the increased amount of MDA and reduced activity of ROS-cleaving enzymes in tissues under such osmotic stress conditions. Conducting experiments in Ca^{2+}-free water medium have induced inability of plants to adapt to osmotic stress conditions and caused dramatic increase in MDA accumulation in plant tissues.
All together our data suggest that adequate adaptation to osmotic stress in plants both dependent on signal perception mechanism that involves PLD enzyme and adaptational mechanisms that is influenced by Ca^{2+} ions by a large scale.

We thank Prof. K. Hirshi for transgenic tobacco plant seeds and Prof. S. Zaika for Maize seeds. This work was funded by the Fundamental Research fund of Ukraine grant № F10/24-2005 and
F14/247-2007.

THE FREE POLYAMINES IN GLYCOPHYTE *PLANTAGO MAJOR* AND HALOPHYTE *THELLUNGIELLA HALOPHILA* UNDER SALT STRESS

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Soil salinity is serious problem in both agricultural and natural ecosystems. Plants are classified as glycophytes or halophytes to their capacity to grow on high salt medium, but most plants are glycophytes which include sensitive, moderately tolerant and very tolerant species. Various possible functions were hypothesized for free polyamines (PAs) in response to salt stress. Indeed, various patterns of PAs endogenous changes, often conflicting, were reported in relation to genotype tolerance, stress nature and duration of stress imposition. In our experiments with two plants – glycophyte *Plantago major* and halophyte *Thellungiella halophila* (*Th.halophila*) were grown in water culture and in age of 6 weeks were treated with 100 mM NaCl during three days. In these plants we studied the dynamic of content of free PAs – putrescine(Put), spermidine (Spd) and spermine(Spm). Constitutive level of Put is comparable in both plants in leaves, but in roots *Plantago major* it was higher then in *Th.halophila.* In the same time stress-dependent accumulation of Put was not observed in both plants. Thus it was no significant difference between two species in spite of the difference in salt tolerance. The level of Spd in *Th.halophila* remained constant during 24 hours. Than that decreased, especially, in roots (after 24 till 72 h.) In *Plantago major* the decrease of Spd content was observed only during first 24 hours both in roots and leaves, than it returned to constitutive level. *Th.halophila* had the more high level of Spm then *Plantago major*. The stress dependent Spm accumulation in *Th.halophila* occurred after 48 h. In *Plantago major* the increase of Spm level riched the max at 18 h. in leaves and after 72 h in roots.The total level of Spd and Spm was higher in *Th.halophyla*, that correlated with higher salt tolerance. The comparison of expression pattern for gene responsible for PAs biosynthesis enzymes showed that both plants had stable high expression of all investigated gene. Thus the transcription level was not limited or regulated factor in PAs biosynthesis. Probably, the regulation of PAs content was realized by polyaminoxidase or diamineoxidase, the enzymes of PAs oxidation.

Work supported by grant of Presidium of RAS “Molecular and cell biology”.

POLYAMINES AND OXIDATIVE STRESS

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Polyamines are universal organic polycations to be implicated in a wide array of fundamental processes in plants, ranging from triggering cell cycle, genome expression, signaling, plant growth and development to plant adaptation to abiotic stresses.

Stress-induced oxidative stress is one of the early responses to abiotic factors. Oxidative stress is one of the most deleterious effect of environmental stress on plants, which is characterized by accumulation of potential harmful reactive oxygen species (ROS) in tissues: O_2^-, H_2O_2, and $HO^\bullet$. These toxic molecules capable of causing oxidative damage to proteins, DNA, lipids and so on. In our experiments performed *in vitro* was shown, when total DNA, isolated from leaves of halophytic plant *Mesembryanthemum crystallinum,* was incubated in the system generating free radical $OH^\bullet$**,** practically no DNA was detected. The addition of exogenous cadaverine (Cad) or spermine (Spm) to the $OH^\bullet$ -generating system suppressed DNA damage. These PAs inhibited DNA degradation most efficiently at concentrations of 1-5 mM. Numerous reports appeared about stress-induced accumulation of free and conjugated PAs in various plant species. Most important antioxidant properties of PAs are exibited when they form conjugates with phenolic acids. PA conjugates with coffeic, cinnamic, and ferulic acids displayed a higher constant of binding to reactive oxygen species than free PAs. In halophyte *M.crystallinum* all forms PAs conjugates (PCA-soluble and –insoluble were detected). In adult plant the process of CAM induction under salinity is linked with oxidative stress and activation of antioxidant defensive responses. It was found that adult leaves under normal conditions or salinity (400 mM NaCl) contained PCA-insoluble (bound) conjugates of putrescine, spermidine and especially spermine (Spm), which showed a tendency to grow with increased duration of salt action (1.5→ 48 hr). In roots the formation of PCA-soluble conjugates of all PAs, except spermine, was decreased under long-term salinity . A decreased content of conjugated Cad in roots under salinity could explain by the faster oxidation free Cad under salinity. However, the formation of PCA-soluble or PCA-insoluble conjugated Cad was sharply and fast (1,5 h) inhibited by exogenous Cad treatment. This negative effect was removed by exogenous Cad treatment in combination with aminoguanidine (AG), inhibitor of diamine oxidase (DAO). After the treatment of this plant with low concentrations of Cad and Spm (below 1 mM), PAs behaved as antioxidants, whereas high PA concentrations manifested prooxidant properties due active formation of H_2O_2 and increased pH (>7.0) in the apoplast. In this case, PAs facilitated the reverse reaction with the formation of O_2^- from H_2O_2. Thus, inhibiting effect of exogenous Cad on PCA-soluble conjugates formation can be more likely induced by accumulation of O_2^- than H_2O_2.

One of the manifestations of the antioxidant effect of PAs is their ability to regulate the expression of genes encoding antioxidant enzymes. It was found that 1mM Cad added to the nutrient medium for *M. crystallinum* for 2 h induced transcription of the gene for cytoplasmic Cu/Zn SOD form. The addition of the inhibitor AG (1 mM) along with Cad to nutrient medium did not reduced the level of mRNA, which indicates that non-oxidized diamine affected this gene transcription. Root treatment with 1 mM H_2O_2 increased the level of mRNA as well, but to a lesser degree. This supports a previously suggested hypothesis (Kuznetsov et al. 2002) that stress-induced Cad accumulation in the common ice plants and its capability of long-term transport permitted Cad to play a role of a stress signal, which switches on the plant defence mechanism directed, in this case, to the improvement of cell antioxidant activity.

EFFECT OF POTATO PLANT TRANSFORMATION BY YEAST INVERTASE AND Δ12-ACYL-LIPID DESATURASE GENES AT GENERATION OF REACTIVE OXYGEN SPECIES DURING HYPOTHERMIA

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Reactive oxygen species are well known for their signal role during early periods of stress. We suppose that changes in saturation index changes and accumulation of protective material (e.g. sugars) will affect reactive oxygen species status.

Our study was carried out with potato plants (*Solanum tuberosum* L.) cultivars Désirée and Desnitsa. Plants of Désirée cv. were transformed with vector carrying yeast invertase gene under the control of tuber-specific patatin promoter B33 class I, fused with proteinase II inhibitor leader peptide to provide enzyme location in apoplast. Plants were obtained in cooperative work of Max Plank Institute of Molecular Plant Physiolgy (Golm, Germany) and Laboratory of Growth and Development, Timiryazev Institute of Plant Physiology RAS, and gently provided by employees of the last. Plants of Desnitsa cv. were transformed with vector carrying dasA gene, encoding Δ12-acyl-lipid desaturase of fatty acids and thermostable lichenase reporter gene (licBM3). Both genes were placed under the control of constitutve 35S CaMV promoter. Agrobacterial transformation was performed according to method of micro-tuber transformation developed in Departnent of cell biology and biotechnology, Timiryazev Institute of Plant Physiology RAS. Both cultivars were grown in vitro at 22^{o}C under diffused fluorescent light (16 hours a day, LB-80 lamps, 4 klx) on MS nutrient medium, containing 2% of sucrose. Oxidative stress, induced by chilling, was performed by exposition of plants at -7^{o}C during 30 min.

As it was shown in our earlier experiments both transgenic genotypes differed from control plants in their chilling tolerance, assayed by electrolyte leakage and lipid peroxidation intensity. We supposed, that the cause of this effect were generation and accumulation of reactive oxygen species, playing a stress-signal role under hypothermia. To examine this potent case we tested intensity of superoxide anion generation and accumulation of hydrogen peroxide in conditions of oxidative stress, induced by chilling.

Intensity of short-living superoxide anion generation in transformed potato plants of Désirée cultivar exceeded this parameter of control ones. Thus, higher chilling tolerance of transformed plants, described earlier, could be explained to some extent by more developed antioxidant system. This conclusion is supported with data about H_2O_2 accumulation. According to it, transformed plants accumulated more hydrogen peroxide than control under stress conditions, so it could serve as a signal to activation of antioxidant enzymes. After chilling we returned plants into conditions optimal for growth, where decrease of H_2O_2 initial levels were observed in transgenic genotypes, but not in control.

The study is supported by Russian Foundation for Basic Research (project no. 04-04-48476).

PHASEOLUS VULGARIS L. PRIMARY LEAF GROWTH AND ENDOGENOUS PHYTOHORMONES UNDER DROUGHT AND THE EFFECT OF SEEDS TREATMENT WITH ABA

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Plant tolerance to water stress and ability to survive under drought depends on the organism age. The youngest plants are more sensitive to stress but they more easy acquire resistance. Plants treatment with growth regulators can change their tolerance to water stress and recovery after one. The aim of this presentation is to reveal the possible mechanisms of these phenomena at the hormonal level.

Phaseolus vulgaris L.seeds were grown in soil in controlled condition after 3 h imbibition in water or in ABA solution (10^{-6} M). Endogenous phytohormones and anatomy of primary leaf at the stages of maximum mitotic activity (5-th d after germination), cell elongation (9-th d after germination) and growth termination (14-th d after germination) under water deficit were studied by HPLC, bioassays and microscopy methods. Water deficit was induced by cessation of watering during 2 days at all these stages.

Highest levels of zeatin, zeatin ribozide and free gibberellin-like substances (GLS) were determined at the beginning of control plants leaves development. Free cytokinins disappeared and IAA level declined whereas content of zeatin-O-glucozide and ABA increased at the growth termination stage.

Alterations in phytohormones balance were determined under drought at all studied stages. Cell division inhibition and as a consequence decreasing in leaf area were observed as a result of phytohormones disbalance. At the same time the increase of cell thickness and density of palisade tissues took place. The most considerable changes in phytohormones under drought were shown at the stage of maximum mitotic activity of leaf cells: zeatin content decreased 5 times, IAA – 2 times, GLS activity – 4 times. When drought was created at the stage of leaf cells elongation alterations were less essential but 2 fold enhancement in ABA level was shown. *Ph. vulgaris* leaves were the less sensitive to water deficit at the stage of growth termination. Changes in free hormones content were not detected but levels of zeatin-O-glucozide and bound IAA and ABA increased more than 2 times. Phytohormones disbalance was found during long time after rehydratation especially if drought was created at the stage of maximum rate of meristem cells division.

As a result of seeds imbibition in ABA solution increase in leaf thickness and area, formation of the greater stomata amount were observed. Chloroplasts amount did not change. Enhancement in endogenous free and bound IAA and ABA, zeatin, zeatin ribozide, zeatin-0-glucozide, as well as increase in free and bound GLS activity was shown during leaf growth and development. When seeds were treated with ABA solution the tendency to decreasing in stimulating hormones content under drought saved but differences between control and experimental leaves characteristics were less essential and at the stage of leaf maturing they became unnoticed.

Thus, content of zeatin, zeatin ribozide, IAA, ABA and GLS activity declined whereas amount of free ABA increased in *Ph.vulgaris* primary leaf under water deficit. The most considerable changes in phytohormones content under drought were determined at the earlier stages of leaf development. Therefore, the mechanism of plant sensitivity to water stress can be assumed to be connected with reactivity of hormonal system which obviously changes during ontogenesis. Treatment with ABA did not prevent negative effect of water deficit completely but promoted the growth processes normalization.

EFFECT OF PLANT GROWTH REGULATOR – MELAFEN ON PLANT MITOCHONDRIA ENERGETICS

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The plant growth regulator – Melafen was synthesized at the Arbuzov Institute of Organic and Physical Chemistry, Kazan Research Center, Russian Academy of Sciences. The compound is a salt of bis(oxymethyl)phosphine acid. The incubation in the presence of 4 x 10^{-12} M of the compound results in the activation of the electron transport in the respiratory chain of mitochondria isolated from Beta vulgaris sugar beet root. After the incubation, the maximum rates of electron transfer on the oxidation of NAD-dependent substrates increase by 33% and the respiratory control (RC) according Chance increases from 2.3±0.1 to 2.90±0.15. In case of using succinate as a substrate for oxidation, no above effects are observed. By stimulating the activity of NAD-dependent dehydrogenases, Melafen may activate the energy-related processes in cell and provides for a high energy of seed germination. The effect of the compound on the activation of the energy-related processes in cell also is bound with its effect on the electron transfer rate at the end cytochromoxidase site of the mitochondria respiratory chain. The presence of Melafen in the mitochondria incubation medium increases the rate of oxidation of the ascorbate in the presence of tetramethylphenylene diamine (TMFD) from 766.0 ±45.5 to 973.5 ±48.3 natoms O_2/mg mc protein min. It was supposed that the effect of the compound on the energy potential of mitochondria isolated from sugar beet root is adaptive in character. This supposition was verified by experiments carried out in Pisum sativum pea seeds under low moisture conditions. The treatment of pea seeds with a 10^{-7} % solution of Melafen stimulates the shoot growth (18–24% acceleration) both for the control and plants grown under low moisture conditions; the germination of treated and untreated seeds differs considerably. Under conditions of low moisture, the germination of seeds of the control group decreases by 46%; the germination of Melafen-treated seeds almost does not vary. Melafen stimulated the growth of seedling roots under the drought conditions; the effect is of importance for adaptation. Moreover, the presence of 4 x 10^{-12} M in the incubation medium of mitochondria isolated from ageing sugar beet roots activates the alternative oxidase (AO) and has no effect on the activity of this enzyme in mitochondria isolated from roots placed under standard conditions. The activation of AO promotes decreasing the lipid peroxidation processes stimulated by stress factors. Thus, the effect of Melafen on the energy potential of plant mitochondria is adaptive and depends on the mitochondria functional state.

SESSION 7:

Hormones and synthetic plant growth regulators in agriculture

MECHANISTIC MODELS OF OXIDATIVE TRANSFORMATIONS OF THIAMIN AND THIAMIN PHOSPHATES

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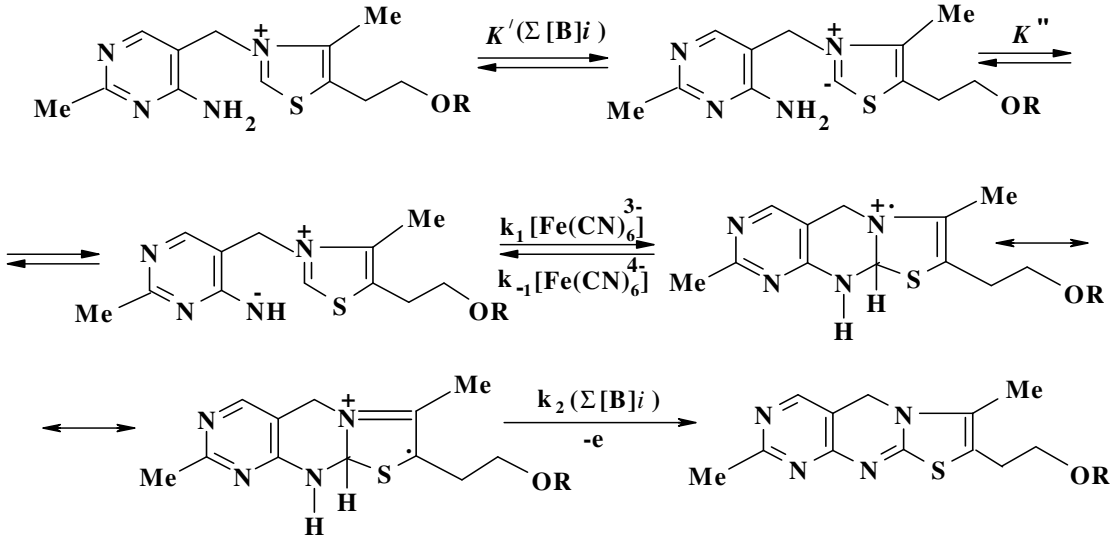
Thiamin (vitamin B_1) is synthesized in the plant leaves and serves of essential metabolic function. The thiamine compound in plant tissue are present in form of unphosphorylated thiamin and in forms of thiamin phosphates. Thiamin diphosphate is the coenzyme of pyruvate dehydrogenase and α–ketoglutarate dehydrogenase complexes, transketolase and other carbohydrate enzymes. In plants, thiamin diphosphate catalyzes also conversion of pyruvate to acetolactate which is included in bisynthesis of branched-chain amino acid.
It is known that thiamin and thiamin phosphates during its functions in living cells in the presence of hydroxide may undergoue oxidative transformations. We have studied the kinetics of model thiamin, thiamin monophosphate and thiamin diphosphate oxidation by ferricyanide to the thiochrome derivatives in phosphate buffer at pH 7.5-8.0. The reaction is inhibited by ferrocyanide. The dependence of reciprocal of the observed pseudo-first-order rate constants on ferrocyanide concentration at determined initial concentration of ferricyanide is linear. In presence of excess of ferrocyanide the reaction is first order in substrate and oxidant concentrations. Analysis of the kinetic data reveals that oxidation by ferricyanide involves one-electron transfer step with thiazolium radical cation formation.



There were determined rate constants $k_1' = k_1 K' K''(\Sigma[B]_i)$ and partition ratios $k_{-1}/k_2(\Sigma[B]_i)$ for oxidative transformations of thiamin, thiamin monophosphate and thiamin diphosphate. It is found that thiamin diphosphate is more reactive substrate in comparison with thiamin and thiamin monophosphate. It is assumed that base-catalyzed oxidation of thiamin and its phosphorylated derivatives serves as mechanistic model of their catabolism in biological systems.

[1] A. I.Vovk, L. V. Babii, I. V.Murav'eva. *Russ. J. Gen. Chem.*, 2002, **72**, No 11, 1808-1812.

GROWTH REGULATION OF CEREAL ROOT ROT AGENT ISOLATES WITH EXOGENOUS PHYTOHORMONES

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Studying the effect of exogenous phytohormones on a plant and phytopathogenes is of certain interest: first, to determine a role of hormonal system of a plant in realization of plant resistance mechanism to a pathogene, and second, to search nontoxic compounds and their concentrations for protecting plants from diseases.

The objective of the work was to investigate the effect of exogenous phytohormones on growth of isolates of cereal root rot agent *Bipolaris sorokiniana* (Sacc.) by Shoemaker and to reveal the dependence of the effect value on pathogenicity of isolates.

The subject of the investigations was monoconidial isolates *B. sorokiniana* isolated from natural pathogen population of Eastern and Western Siberia: strongly pathogenic – 23, 53, 410, 421, 451, 824, 830; medium-pathogenic – 63, 150, 211, 233; lightly pathogenic – 260, 648. Three runs of experiments were carried out; the replication of tests was fourfold. The results of the experiments were statistically processed with using STATISTICA 6.0 software package.

It was established that phytohormones introduced into nutrient medium in concentrations being approximate to their contents in plants changed cultural-morphological characters and growth parameters of pathogen's isolates in the Chapek's agar medium. The fungus colonies were distinguished by greater compactness, decrease of edge irregularity, and higher mycelium; sporification came earlier by 1-2 days than that in the control did. These particularities were typical for the pathogen growth in the presence of all the hormones (GA, IAA), but were most pronounced in the kinetin media. As a result of the investigations carried out, it has been established that monoconidial isolates *B. sorokiniana* can be divided as to growth character in the Chapek's agar into two groups: fast-growing (150, 260, 421, 451, 648, 624, and 830) and slow-growing (63, 211, 233, and 410) ones. The linear growth velocity of fast-growing isolates made up 10-18 mm/day during the first 3-4 days of cultivation and 1-3 mm/day by the end of cultivation; the diameter of colonies amounted to 70-87 mm. The growth velocity in the kinetin media was lower and made up 6-10 mm/day in the beginning of cultivation and 2-3 mm/day in the end. The growth velocities of slow-growing isolates in the control and the experimental variants did not differ for certain and made up 4-7 mm/day; with that, the diameter of colonies amounted to 60-78 mm by the end of cultivation. We revealed the correlation between a degree of inhibition of linear growth with kinetin, growth velocity in the Chapek's agar, pathogenicity and phytotoxicity of their metabolites – the coefficient of rank correlation made up 0.96.

RESEARCH THE PLANT GROWTH STIMULATING ACTIVITY AND PHYTOHORMONE CONTENT IN THE PREPARATION AVERCOM OBTAINED FROM *STREPTOMYCES AVERMITILIS* UCM Ac-2179

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Use in practice the natural plant growth regulators which reveale growth stimulating activity and raise the plant resistance to the stress factors, first of all to the pathogens, is the perspective element of modern plant protection system. The great attention at this situation is spared for working out the complex plant growth regulators created on the base of natural raw materials which include such components as pytohormones, vitamins, amino acids, fatty acids, and other physiology active matters.

It was known lately about streptomycete ability for synthesis of complex antibiotic avermectin with insecticidic, acaricidic, nematocidic and insignificant fungicidic action. Some preparations were created on the base of avermectin and used for pest control: pytoverm, aversectin C, ivermectin, actophyt etc. Information about plant growth stimulating properties of these preparations are absent in literature.

Collaborators of the General and Soil Microbiology Department of IMV of NASU isolated and selected *Streptomyces avermitilis* UCM Ac-2179 - producer of avermectin. It was created the new complex microbial preparation avercom on the base of this antibiotic. Previous researches have shown high nematocidic activity to gall nematode *Meloidogyne incognita* – the causative agent of meloidoginosis, the disease of vegetable culture root systems. It, in the future, will allow to use the avercom as antiparasitic mean for phytoparasites. Studies of avercom physiological activity with specific biotesting have shown its ability to stimulate wheat, oats, rape, radish, cucumbers, and tomatoes germination, energy of sprouting and sprout development.

It has been studied, in this connection, the *Streptomyces avermitilis* UCM Ac-2179 ability to form phytohormones. For this purpose, the streptomycete strain was grown in the full value soy-bean and synthetic media. Phytohormones were calculated at the ethanol extracts from microbe biomass quantitative spectro-densitometric thin layed chromatography.

Results have testified the *Streptomyces avermitilis* UCM Ac-2179 capacity for synthesis auxins and cyitokinins at the both media. Auxins were represented by indolilacetic acid (1105.14 and 2094.56 nano-g per 1g of dry biomass accordingly above mentioned media); cytokinins were represented by *iso*-pentyladenin (2182.04 and 837.82 nano-g/g), zeatin (759.67 and 168.87 nano-g/g), and zeatin-riboside (906.61 and 4228.36 nanog/g). The difference in quantitative production of phytohormones by streptomycete grown in the various media may be connected with existence of some precursors in the soy-bean medium. At the other hand, avercom also is rich in phytohormones (nano-g per ml): indolilacetic acid 216.61, *iso*-pentyladenin 427.68, zeatin 148.9, and zeatin-riboside 117,9.

Thus, we at the first demonstrated the ability of *Streptomyces avermitilis* UCM Ac-2179 to form some phytohormones and obtained the preparation avercom with phytostimulating action which was depended on presence of auxins and cytokinins in its content. The strain *Streptomyces avermitilis* UCM Ac-2179 may be used in future not only as producer of nematocidic preparation, but also for creation of new microbial complex preparations with plant growth stimulating action.

ANTIOXIDANT POTENTIAL OF STABLE NITROXIDE RADICAL CLASTERED BY A RESORCINARENE PLATFORM

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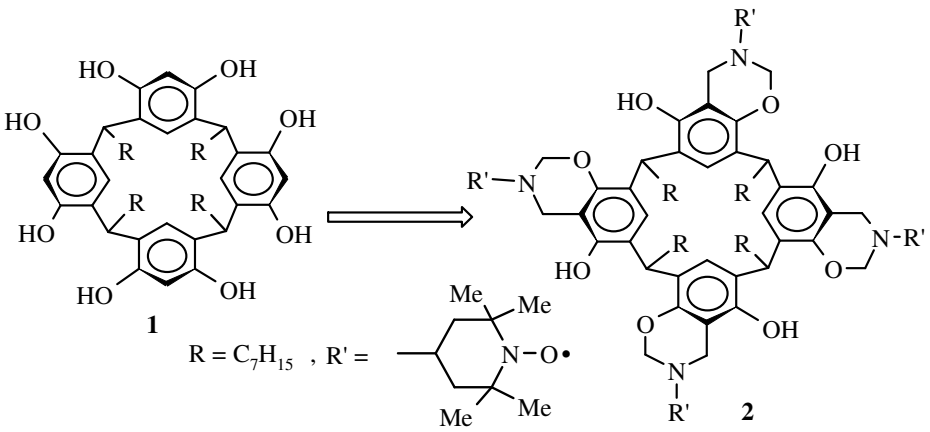
Highly reactive oxygen species and lipid peroxyl radicals are produced in living cells as a result of aerobic metabolism and are involved in a number of life sustaining biochemical processes. The failures of the antioxidant protecting systems initiated by various factors results in oxidative stress causing a number of oxygen radical-derived pathologies. Therefore, there is a considerable demand in new antioxidants and antiradical agents, which may be used for *in vivo* scavenging of free radicals. We have reported that preorganization of several biomimetic groups on the macrocyclic platform such as calix[4]arene results in highly active enzyme inhibitors [1,2]. We have assumed, that preorganization of four antioxidant fragments such as free radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) at the wide rim of macrocyclic resorcinarene **1** [3] was expected to result in an enhancement of antioxidant activity.



The radical scavenging abilities of C_4-symmetrical tetraoxazine derivative **2** were evaluated by effects on superoxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Superoxide mimic activity of spin labeled resorcinarene **2** was found to be may two more efficient compared to 4-hydroxy-TEMPO. DPPH scavenging ability of these macrocyclic compounds in term of stoichiometry is more than two orders of magnitude higher than for resorcinol. The results indicate that spin labeled resorcinarene **2** also effectively suppress the linoleic acid peroxidation in micelles in presence of free radical initiator. The screening test to determine the peroxyl radical-trapping efficiency of compounds tested includes examine of influence of inhibitor on conjugated dienes formation from linoleic acid in micelles in presence of 2,2'-azobis(2-amidinopropane) as the initiator. Compound **2** was roughly an order of magnitude more active compared to Trolox C, known inhibitor of lipid peroxidation.

1. [1] A. I. Vovk, V. I. Kalchenko, S. A.Cherenok, V. P. Kukhar, O. V. Muzychka, M. O. Lozynsky. *Org. Biomol. Chem.*, 2004, **2,** No 21, 3162-3166.
2. [2] S. Cherenok, A. Vovk, I. Muravyova, A. Shivanyuk, V. Kukhar, J. Lipkowski, V. Kalchenko. *Org. Lett.,* 2006, **8**, No 4, 459-552.
3. [3] L. Kröck, A. Shivanyuk, D. B. Goodin, J. Rebek. *Chem. Commun.*, 2004, No 3, 272-273.

SYSTEMIC APPROACH AND APPLICATION OF NATURAL PRODUCTS FROM *RRHODIOLA ROSEA* L. AS PLANT GROWTH REGULATORS IN AGRICULTURE

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All biosystems are open systems; driven by complex energy transfer systems (*metabolic system*) and controlled by intricate regulatory (*genetic and physiological regulation*) mechanisms. Each functioning biosystem must adapt to and, to a certain degree, posses mechanisms or means to control both intra- and extra-organism conditions, "*purpose*". Three *purposes* of different hierarchical importance are: 1) provision of the stationary non-equilibrium state (*the first order purpose*); provision of the constant internal environment (*homeostasis - the second order purpose)*; acquirement of high level of functioning (*the third order purpose*). Under adverse conditions, the biosystem must adapt to its environment. The organism must have the capabilities to change itself or the external environment, including community function, to achieve the purpose of highest possible level. Living systems are *adaptable*, that means that they have a capacity to change physiological processes in a direction that leads to diminishing or avoidance of the full impact of the stress. It is important to point out that the adaptations do not lead to optimization; they only improve the maintenance capacities of biosystems. An important step in understanding the nature and mechanisms of their influences on biosystems appeared after introduction in 1947 of the concept of adaptogens by Lazarev. He defined them as substances meant to put the organism into a state of non-specific heightened resistance in order to improve resistance to stresses and to adapt to extraordinary challenges. As in the case of stress, the conception of adaptogens was initially developed to explain the protective effects of some natural products on human and animal systems and was later extended to include their effects on all biosystems. There are well known parallels between the action of some substances and occurrence of disease resistance in plants and animals. The most impressive example of such substance is salicylic acid (*SA*) and its derivatives, isolated from extracts of different plants. It appears to have multiples modes of action since exert a wide range of clinical effects including reduction of pain, fever, inflammation, blood clotting, and the risk of heart attacks and strokes. Exogenous supplied *SA* has been shown to affect a large variety of processes in plants, including stimulation of stoma closure, seed germination, fruit yield and glycolisis. The strongest of known plants containing adaptogens is *R. rosea*. The most important detected compounds are tyrosol, its glycoside salidroside, and phenylpropanoids glycosides: rosavin, rosarin, and rosin. Rhodiola has been used in traditional folk medicine. In our experiments they demonstrated high antioxidative activity. Plantlets of different species of higher plants obtained from the seeds sprinkled with the extracts from *R. rosea* had a small tendency to produce more developed roots system and less developed shoots in comparison with those obtained from control seeds. Under the influence of heat shock the growth of the roots of the plantlets obtained from seeds untreated with extract was stopped completely and those obtained from seeds sprinkled with extracts continue to grow at the level comparable with that of control plants. The extract of *R. rosea* could be considered to be an adaptogen for plants. Under its actions the resistance of plants to heat shock increased. In supplemental experiments, it was shown that the beneficial effects extracts from *R. rosea* was partially dependent upon its protective action and as well due to cellular and molecular events immediately after heat shock. Thus the system theory helps biological researchers in analyzing a complex experimental results or designing new experiments. It is apparent that utilization of natural products by biosystems could be regarded as external signals. The effect of each external signal is determined by the "*target*" of its action, which is similar to action of plants hormones.

The research described in this work was made possible in part by the INTAS project 05-104-7603.

REGLALG, A NEW PLANT GROWTH REGULATOR WITH PERSPECTIVE OF UTILIZATION IN ORGANIC AGRICULTURE

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Plant hormones are in the center of the scientists' interests because they are implicated in regulation of plants growth and development and could be utilized for specific regulative effects on plants in order to obtain desired practical results. Hormones demonstrate biological activity in very low concentrations, usually at concentrations 10^{-9} – 10^{-6}M. Plants produce hormones naturally, while humans produce ***plant growth regulators*** **(*PGR*)** synthetically or extracting them from living organisms. Native *PGR* could contain hormones and other substances that demonstrate biological activity at concentrations comparable with those of hormones. *PGR* are specifically applied to single plant or plantation to achieve the desired biological and agronomical response. Apart from growth regulating properties plant growth regulators have positive impact on plant responses to biotical and abiotical stresses. Plants elaborate a number of inducible defenses including production of antibiotics, phytoalexins, developing of hydrolytic enzymes, toughening of the cell wall, and changing of organs and tissues growth rate. Altogether these changes cause acquisition the plants resistance to different stress factors by the mechanisms, named ***systemic acquired resistance*** **(*SAR*).** Some *PGR* could induce *SAR*. The very large amount of chemical *PGR* is detrimental for the environment and human health and needs to be replaced by less detrimental compounds, preferably from natural sources. Plant intrinsic responses to stress factors can be induced to attain a wider, more durable resistance, including the *SAR*. Although mentioned phenomena are complex and our knowledge of them incomplete, this is an area of enormous promise in plant protection. We have obtained a preparation ***Reglalg***. The natural PGR ***Reglalg***, extracted from algae in special conditions, could promote the solving of the mentioned problems. Used for treating of winter wheat seeds before sowing, ***Reglalg*** has been shown to promote vigorous root system and detain shoots growth. Its components acts as signal transducers and activate defense response in plants. These influences leads to increase plant resistance to frost, drought, high temperature and snow mould, acting as an inducer of *SAR*. It assures the development of vigorous plants, with longer period of vegetation that led to augmentation of plant yield by 8-23%. Active components of the preparation ***Reglalg*** are in specific composition and dissolved in ethyl or butyl alcohol. The solution is not toxic for human beings or animals. It is utilized for seed treatment before sowing. In combination with fungicides and pesticides ***Reglalg*** promote their activity. As a result, the detrimental effect of chemical crop protectants on the environment and human health could be reduced, while the efficient use of natural resources improved. It can be applied using the techniques developed for the chemical compounds. Implementation is thus not hampered by the requirement of costly investments or unfamiliarity of the end-users. The natural origin, large spectrum of biological activity, ease and safety of application, and also high efficiency, are those unique properties which cause perspectives of the preparation ***Reglalg***. It is certified for utilization in Moldova agriculture.

The research described in this publication was made possible in part by the Award STEP 621 of MRDA/CRDF. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect those of the MRDA/CRDF.

PHYTOHORMONAL STATUS CHANGES IN DIFFERENT BY RESISTANCE TO THE *FUSARIUM* INFECTION WINTER WHEAT CULTIVARS AT PATHOGENESIS

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Phytohormones of pathogenic fungi in the relationships with the host plant stand out as the inductors of physiological processes and undoubtedly are the important link at the formation of specific and nonspecific adaptation reactions. Literature data indicates phytohormonal balance changes in the wheat -necrotroph *Septoria tritici* system. But it is still under the discussion whether these changes are directed to the enhancement of pathogenesis process or to the resistance induction.

We have studied the phytohormonal content changes in the seedlings of two winter wheat cultivars differ in resistance degree to *Fusarium graminearum* at the infection with this pathogen. It was established that plants; infection is resulted in the decreasing of both IAA and ABA content in the seedlings of persistent cultivar (Columbia), while the IAA content in the seedlings of inconstant cultivar (Bilotserkivska napivkarlikova) is increased dramatically but the ABA content remains on the control level. Similar mechanisms were revealed at plants infection with the fungi of *Septoria* family. It is concluded that IAA in the infected plants is most likely of the fungal origin and the increase of its contents in the plants inclined to the infection cause certain changes in the cell structures which facilitates fungi penetration. The same conclusion was made by the Maksimov I.V. at the experiments with the wheat tissue culture cultivated with the *Tilletia caries* pathogen.

In our experiments the level of zeatin has slightly increased in the wheat plants of persistent cultivar Columbia and has not changed in favorable cultivar. In the first case the increase of zeatin is probably caused by the decrease of its transport form -zeatin-rhibozide. Maksimov I.V. has revealed the increase of cytokinin content in two wheat cultivars persistent and favorable to *Tilletia caries* pathogen although the cytokinin content in persistent cultivar was noticeably higher then in the favorable one. Though the fungi of *Septoria* family like the *Fusarium graminearum* (in our experiments) are necrotrophs but *T. caries* fungi is biotroph, the initial plants' reactions on phytopathogenie infection are, probably, the same.

First of all it refers to the phytohormonal system reactions and metabolic processes concerned with its functioning. Thus, the anion chitin-specific peroxidase forms were found in persistent to *Septoria tritici* blotch wheat cultivar Diamant which interact with the chitin of phytopathogenie fungi and activate synthesis of lignine in site the pathogenic fungi structure location. Besides, anion peroxidases can directly bound with chitin in sites where the activity of enzyme is not inhibited and IAA promotes reduction of both general peroxidase activity and its specific isoforms. There are evidences of cytokinin's participation in signal transduction under the different stress conditions. Also cylokinins take pan in the expression *o*\~ various defensive genes and induce synthesis of defensive compounds alkaloids.

Activation and deactivation of defensive mechanisms (in case of favorable cultivars) at pathogenesis depends both on phytohormonal concentration and their interaction with other physiologically active compounds. The contents and correlation of phytohormones might change depending on both the plants and fungi developmental stages. Undoubtedly, phytohormones' interaction in the investigated plants is the important factor that controls mutual relations between plant and pathogenic fungus.

NEW CHEMICAL GROWTH FACTORS OF PLANTS

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New chemical growth factors on plants cellular cultures of wheat and a potatoes are studied. Their biological activity on the germinating seeds of wheat is revealed, effective concentrations for regulating of increase and development of plants and cellular cultures are determined. Strengthening of wheat and a potatoes cellular cultures proliferation, and also stimulation of germination and acceleration of development shoots and roots from 2 wheat genotypes seeds has been shown.

The new chemical compounds derivatives of pyperedols are recommended for using as plants growth regulators for the plant biotechnology, the plant growing and the agriculture.

Work is executed within the framework of the State program «Development of Space activity in Republic Kazakhstan during 2005-2007 years».

Employees of A.B.Bekturov Institute of Chemical Sciences synthesized new potential biologically active compounds (aryl-substituted piperidin-4-ones and 1,2,5-threemethyl-4-dimsilpiperidin-4-ol) which have been tested as growth factors on wheat and a potatoes cellular cultures.

A series of experiments on cellular cultures in vitro conditions where in skilled variants in structure of nutrient mediums entered studied substance in different concentration is lead and base procedures were used as the control. Callus formation on explants from a potatoes and wheat leaf tissues occurs at addition to Murashige-Skoog culture medium (MS) 2,4-D (2,4-diclorophenoxyacetic acid); regeneration of shoots begins after the transfer of callus tissue to MS medium with the addition of cytokinin - 6- benzylaminopurine (BAP) on 4-5 week of cultivation. An increase in the shoots and roots during the microclonal multiplication of potatoes occurs on the Gamborg's B-5 medium in the presence of kinetin. Conditions of the cell and plant cultivation in vitro: the temperature of air in the thermostat and light-cultural klimokameras - 24-26°C, 16-hour photoperiod, additional illumination, the duration of cultivation - 4-6 weeks.

In the experiments under the conditions in vitro determined the frequency of the calluses formation of wheat and potatoes, increase and volume of calluses, the appearance of rudiments of roots, shoots and they measured on the seeds of wheat: a quantity of overgrown seeds, the height of shoots, a quantity of leaves and roots; the length of roots.

Vegetal experiences in vivo were the sprouting of the wheat seeds in the solutions of the studied substances. As control served the seeds were processed by water. Seeds of the 2 genotypes set down into the Petri dishes on 100 pieces in the 3rd of multiple repetitions. The germinating capacity of seeds was determined on the 3d, 6th and 9th day of germination.

Experimental data showed that on increase in the biomass of the wheat and potatoes cells the greatest influence showed the substances under the cipher A-1 and AYE - ץ, to an increase in the stems of wheat - A-1, AYE, AYEYAK and Kazakhstan -4, and to an increase in the roots - KN -10, Kazakhstan -4 and AYE.

PERSPECTIVES OF GIBBERELLIN-PRODUCING PSEUDOMONAS RHIZOBACTERIA APPLICATION IN AGRICULTURE

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Gibberellins synthesizing by plants, bacteria and fungi are natural hormones with practical applications in agriculture and brewing. Commercial preparations based on hormones of this group, for example, gibberellic acid are used to increase different grape and citrus sorts productivity and processing of strawberry plantations. Besides gibberellins are used in potato growing for plants moving from dormancy, to increase vegetative weight in lea management and enhance of tannins content in tea leaves.

It is known that the inoculation of several plant species with various strains of *Azospirillum*, *Rhizobium* and *Pseudomonas* bacteria often leads to enhancement of growth and yield, and among the possible mechanisms which have been proposed to explain this effect is production of plant hormones by the bacterium.

Earlier we have selected *Pseudomonas aurantiaca* B-162 strain synthesizing a large quantity of phenazine antibiotics and capable to stimulate plant`s growth. On the basis of B-162 by means of chemical mutagenesis we have received B-162/498 strain, which production of antibiotics was 3 times above than the efficiency of initial strain. *P. aurantiaca* B-162/498 strain also stimulated plant`s growth.

It was established, that seeds processing with *P. aurantiaca* bacteria stimulates growth of seedlings (in 1,4-2,2 times) and root system (in 1,7-3,3 times) of various agricultural plants (cucumber, beet, cabbage, tomato, carrot and radish). Because of the intensive development of *P. aurantiaca* in rhizosphere it might be expected that maximum amounts of phytohormone would be produced therein. We have shown that both strains synthesize equal level of auxin indole-3-acetic acid (6,54±0,23 mkg/ml), that is characteristic for rhizospheric bacteria. At the following stage we defined quantity of gibberellins producing by B-162 and B-162/498 strains. The bacteria were grown in 250-ml flasks containing 50 ml of the M9 medium at 28ºC in the dark during 48 h. Gibberellins concentrations in the media were determined by fluorometric method. A 0.2 ml aliquot of culture medium was shaken with 0.2 ml of 96% ethanol and 2 ml of mixture of equal volumes of sulfuric acid and ethanol. Than mixture was incubated at 48ºC for 30 min and the fluorescence emission at 464 nm was measured (excitation at 406 nm). It was established, that B-162 strain produce 13,18±0,34 mg/l of gibberellins while productivity of B-162/498 strain reach to 20,49±0,84 mg/l. It is known that other strains synthesis smaller amount of gibberellins (for example, productivity of *Pseudomonas fluorescens* is 2 mg/l, *Rhizobium radiobacter* – 4 mg/l, *Bacillus subtilis* – 12 mg/l) that allows to consider received *P. aurantiaca* B-162/498 strain as potential object for use in agriculture for plant`s growth stimulation and yield enhance.

EXPLANT DENSITY AND MORPHOGENETIC, PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF *IN VITRO* BUCKWHEAT HYPOCOTYL CULTURE

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Qualitative and quantitative composition of the substances secreted by cells and tissues in culture medium depends on cell culture density. Some of these substances can influence on morphogenesis, proliferation activity and physiological parameters of cells and cell culture *in vitro*. The aim of this study was to estimate the influence of explant culture density on the induction and formation of proembryonal cell complexes (PECC) and some process characteristics (medium pH, biomass yield, extracellular proteins) in the experimental system of buckwheat hypocotyl explants (*Fagopyrum esculentum* Moench.).

In experiments we used hypocotyl segments of 4day old etiolated seedlings. The different explant densities were tested: 10, 20, 40 and 80 explants per 20 ml of culture medium. Explants were consistently cultured on the liquid media: medium **I** supplemented with 8.0 mg/l 2, 4-D (1 week), medium **II** containing 8.0 mg/l 2, 4-D (4 weeks).

The transfer from medium **I** to medium **II** stimulated the development of proembryonal cell complexes in explant tissues. A cell suspension formed at the same time with PECC during explant cultivation. The maximum number of explants with PECC (almost up to 100%) was observed at culture density 10 and 20 explants per 20 ml. The largest dry weight of the suspension biomass (200% of initial explant weight) was observed at explant density 20, the smallest biomass yield (100 % of initial explant weight) – at culture density 80 explants per 20 ml.

The effect of explant density on medium pH was also investigated in this study. Acidification of the medium **II** occurred at all culture densities. The greatest decline of pH value (up to 4.8) was observed at culture density 20 explants per 20 ml. A higher pH (4.95-5.0) was observed at the density 4 and 80 explants per 20 ml.

The electrophoresis of extracellular proteins showed some differences between medium **I** and medium **II,** but the negligible difference between media with different explant density was revealed.

We connect the observed changes with extracellular substances extracted by explants and cell suspension formed during the cultivation.

CYTOKININE SECONDARY HORMONE FROM WHEAT SEEDS EMBRYOS: PURIFICATION, PROPERTIES AND APPLICATION

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It was shown that cytokinine causes the formation in wheat seeds embryos the cytokinine secondary hormone (CSH).

We have developed the method of purification of CSH which includes hydrophobic chromatography on column with octylsepharose 4B and reverse-phase chromatography on column type RP 18. High purified CSH by its properties is very close to fusicoccine. The CSH shows the high cytokinine physiological activity at concentration hundreds times less and three times quicker than cytokinine itself. So it causes the formation of amarantin in the amaranthus seedlings, prevents the ageing and yellowing of isolated leaves of *Singonium auritum* and causes intensive growth of bosom buds and formation of new steams and leaves from the decapitated main steam of *Phaseolus vulgaris* and *Impatiens balsamina.*

CSH has its own physiological and biochemical properties, so in contrast to cytokinine CSH causes the formation of main and lateral roots of steams cuts and isolated leaves.

It was shown that CSH increases the activity of ATPase of plasmatic membranes which were isolated from roots of wheat seedlings and from wheat seeds. The activity of this enzyme is specific to calcium ions but not specific to potassium and sodium ions.

It was shown that CSH activates the NADP-GDh only in the spherosomes which located in nonembryonic part of wheat grain.

Also we developed new methods for wide application of CSH in agriculture and forestry and ecology.

CSH is very perspective for vegetative duplications of trees and bush plants for example for such as *Azalea*, rose, lemon, *Tamarix Ramozissima, Caragana arborescens, Elaeagnus angustifolia*. The CSH is very interesting for ecology because it increases the adaptation to stress conditions: salinity and winter hardiness. Some tens milligrams of CSH per 1 hectare give 33% increasing of yield of winter wheat.

THE USE OF PLANT GROWTH REGULATORS FOR ELABORATION OF NEW MICROBIOLOGICAL BIOTECHNOLOGIES

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Bacterial preparations on the basis of nitrogen-fixing and phosphorus-solubilizing microorganisms arc characterized by the phitostimulating properties, they also play an important role in provision to plants with ecologically pure biological nitrogen and improve to optimum phosphoric nutrient. Antiphrastic microbial antibiotics on the basis of avermectines with insecticidal and anthelmintic activity are considered as the most perspective among biopreparations. As a result the bacterial preparations promote the increase of agricultural crops and improvement of its quality.

The goal of our investigations was development of biotechnological methods of the microbial preparations intensifying with plant growth regulators (PGR). Objects of investigations were microorganisms selected in IMV NASU: *Bradvrhhphium jponicum* UCM B-6035 (basis of the preparation nitragine); *Bacillus megateriumlM¥* B-T^SS^nasis of the preparation phosphobacterine); *Streptomyces avermitilis* UCM Ac -2177 producing avermectine complex (basis of the preparation avercom). Such PGR as emistime C, agrostimuline, eney and ivine, developed in the Institute of Bioorganic Chemistry and Petrochemistry of NASU and STC "Agrobiotech" were investigated.

Cultivation of biotechnologically important strain *Bradyrhizobium japonicum* UCM B-6035 with ivine, agrostimuline, or eney allows to raise considerably the synthesis of biomass soya rhizobial bacteria. The most of a microbial biomass which exceeded parameters of the control over 2,2 times was observed at addition of ivine in concentration 0,1 πl of preparation per 1 ml of nutrient medium. *B. japonicum* UCM B-6035 biomass was increased at eney action amount of 74,3-91,4, at agrostimuline - 31,2-57,3% from control (without PGR).

In experiments with *B. megaterium* UCM B-5724 was shown, that at preparation per 1 ml of nutrient medium with emistime C or eney in concentration 0,1 πl preparation per 1 ml of nutrient medium, the specific growth rate of bacteria was increased on 14,9 and 8,5% accordingly, the maximum biomass was on 20,1-34,7 % more, than in the control. The important biotechnological characteristic of the strain is its ability to decompose the soil organic phosphorus compounds and to transport phosphorus in the form assimilated for plants. The culture, grown up on the medium containing eney in concentrations 10 or 0,1 πl a preparation per 1 ml of nutrient medium showed the highest phosphatase activity witch exceeded the control over 1,7-2,4 times.

It is necessary to add, that the culture which has been grown un at PGR presence, got higher resistence to action of chemical factors: pesticides, respiration inhibitirs and oxygenated water. It could be supposed that such cultures will be stable in unfavorable environment conditions.

Researches of PGR influence on the ability of avermectine complex producer *Streptomyces avermitilis* UCM Ac -2177 to produce the avermectine complex have shown that the maximum avermectine accumulation was marked in a variant with ivine in concentration of 100 πl a preparation per 1 ml of nutrient medium and consisted 1225 mkg/ml that on 26% is more than in the medium without PGR. Anthelmintic action was increased.

Thus, the addition of PGR into a nutrient medium for cultivation of agricultural important microorganisms in optimum concentration for each strain can be recommended as a new biotechnological procedure promoting increase of microbial biomass synthesis and of its physiological activity.

METABOLIC ASPECTS OF METHYLOTROPHIC BACTERIA INTERACTION WITH PLANTS

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Aerobic methylotrophic bacteria (methylobacteria) and methanotrophs are widespread in nature and appear to be tightly connected with the plants. Earlier, such a connection was shown mainly for the pink-pigmented facultative methylobacteria (PPFM). Our studies on 140 species of mono- and dicotyledonous plants have shown that their seeds, phyllo- and rhizosphere are colonized by taxonomically different methylobacteria belonging to *Xanthobacter, Paracoccus, Methylophilus, Methylobacillus, Methylobacterium*, and also by methanotrophs of the *Methylocystis* genus. Remarkably, we found methylobacteria and methanotrophs on the surface and inside tissues that provides for these methylotrophs better survival at low and high temperatures or during drought. Alternatively, under favorable weather conditions followed by active plant growth and metabolism accompanied by emitting of volatile C_1-compounds, the methylotrophs form biofilms on the leaf surface, thus preventing the C_1-volatiles evaporation to the atmosphere.

Hence, it logically follows that methylotrophs not only colonize plants but are symbiotically related to them. By using TLC, HPLC, MS and bioassays, cytokinins and auxins (up to 120 μg/ml) were detected in spent media of methylobacteria and methanotrophs belonging to different taxa. PCR analysis revealed the presence of nucleotide clusters homologous to the *ipt* genes responsible for cytokinin synthesis in the genomes most of tested methylotrophic bacteria.

Enzymic analysis and identification of the intermediates showed that these bacteria synthesize indole-3-acetic acid (IAA) via indole-3-pyruvic acid (IPvA). The *M. extorquens* gene RMQ09094, named *bfd*C (**b**enzoyl**f**ormate **dec**arboxylase) was amplified and cloned into plasmid pET-22b(+) (Novagen). The superproducer of BfdC was obtained on the basis of *E. coli* BL21 (DE3, pT-GroE). The cell culture of *E. coli* BL21(DE3) with induced protein BfdC from *M. extorquens* AM1 produced four times more indole compounds than that of *E. coli* BL21 being transformed by plasmid pET -22b(+). We also obtained *M. extorquens* with deleted gene *bfd* which synthesized three fold less indole compounds than the parental strain, thus indicating that BfdC is responsible for the key reaction in IAA biosynthesis, i.e. decarboxylation of indole-3-pyruvate. Hence, we first proved the presence of the key enzyme of auxin biosynthesis in methylobacteria and a bifunctional protein BfdC is involved in this process.

Finally, we first demonstrated a stimulatory effect of the methylobacteria and methanotrophs on the *in vitro* growth and morphogenesis of colonized tobacco plantlets (*Nicotiana tabacum* L.) and wheat cells of immature embryos (*Triticum aestivum* L.). Such colonization gave a stable plant-methylotrophic association and resulted in a higher growth rate of the plantlets, their enhanced regeneration potential, and tendency to rooting. The colonized transgenic tobacco plantlets expressing the agrobacterial cytokinin gene *ipt*, have restored the rooting ability. These results implied the promising use of methylobacteria and methanotrophs in modern plant biotechnology.

This study was supported by the grant RFBR OFI-c 07-04-13559.

MODULATION OF IAA-OXIDASE ACTIVITY OF WHEAT ANIONIC PEROXIDASE BY CHITOOLIGOSACCHARIDES

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In recent years there have been emphasized ecological safety and possibility to induce plant protective potential of preparations that stimulate the resistance of plant cells to different environmental factors. Mechanisms of realization of their biological activity have been widely discussed up to now. One of such compounds which are actively used to strengthen the plant resistance to unfavorable environment is chitooligosaccharides (COSs). It is known that they stimulate the formation of bean grafts on the rootlets. They can influence on the activity of some enzymes, including the anion peroxidase. The aim of this research is to study the putative biochemistry action mechanisms of COSs and to offer some possibilities of the applied method for study the action of other biogenic preparations.

The influence of the COSs on oxidation kinetics of wheat IAA anion peroxidase has been studied. There have been determined catalysis constants rates in the interval of pH 4,2 – 8,0. It is found that the COSs decreased the IAA oxidation by anion peroxidase. The correlation analysis of initial speed dependence in double reverse Laynewer – Berk coordinates enabled to find the inhibition nature changing in accordance with pH and COSs concentration. At pH 4,2 – 6,0 incomplete partially uncompetitive inhibition type appeared not fully, while at pH 4,2 – 8,0 it became purely uncompetitive. The conjugation of IAA with the enzyme at pH 4,2 – 8,0 was degraded 9 and more times, that means that the COSs presence lowered the conjugation of IAA with the enzyme. It was proves that the chitin oligomers competes with the IAA for the conjugation with the protein.

It was found that the conjugation of IAA with the wheat anion peroxidase was depended upon the medium pH. At the IAA oxidation inhibition constant of IAA oxidation increased three fold and more when pH growing from 4,2 to 8,0, i.e. when pH-increased the conjugation of the COSs with the protein decreased.

Furthermore there have been found that the IAA also can create the polyelectrolitic complex with COSs that was observed at changing of IAA absorption spectrum at the presence of the COSs. Probably, this complex have less affinity to enzyme than IAA. It might be also one of the IAA oxidation inhibition mechanisms.

The achieved results show the great role of COSs as an elicitor of protection reaction in regulation of IAA level in the plant cell. Probably due to this fact COSs can influence on the plant growth and differentiation and they can determine plant resistance to unfavorable environmental factors.

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

THE INFLUENCE OF EMISTYM C AND REACOM ON SEDGE (*CAREX HIRTA*) PLANTS UNDER OIL POLLUTION

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On sites where heavy oil pollution has taken place, vegetation is usually scarce or absent. The object of investigation –sedge, *Carex hirta*, a pioneer species on oil polluted soils which together with other biotic and abiotic factors cause the decline of hydrocarbons amount in contaminated soil, especially during the first year after oil pollution. The search of ways of optimization of growth and development of these plants will promote phytoremediation of oil polluted soils.

In our researches we used the emistym C and reacom preparations as possible factors of optimization of plant growth on oil contaminated soils, which are characterized with deficiency of nutritive elements for plants. Emistym C is the product of endophytic fungi, it contains the balanced complex of phytohormons of cytokinine and hybereline nature, amino acids, sugars, peptides, unsaturated fatty acids and microelements. Reacom is a composition of chelate forms of microelements and balanced amount of the NPK active forms. We have analyzed the morphometrycal indexes and measured of sum of chlorophylls (*a+b)* and their correlation in sedge plants under the action of oil contamination, emistym C ($1x10^4$; $2x10^4$; $1x10^3$) and reacom (dilution $5x10^4$).

Oil in concentration 50 ml per kilogram of soil repressed the size of Carex hirta plants. The size of above-ground part of plants from polluted pots was 1,7-fold lower than in control. The growth parameters of plants from contaminated soils under the emistym C actions in $2x10^4$ dilution reached the level of control plants. Our results show, that reacom does not have a positive influence on the growth parameters of above-ground part of sedge plants. Compatible use of reacom, emistym C and oil showed that the inhibiting influence of oil was taken off only under the actions of reacom + emistym C in dilution $2x10^4$. In this variant length of above-ground part was 20% and width of leaves –10% more than in oil variant.

For phytoremediation of soils from oil contamination by a sedge plants large value has the state of underground part of plant – rhizome. It was shown, that plant root system for actions of reacom essentialy changed. Rhizomes diameter and the amount of roots on them increased. Diameter of rhizomes of *Carex hirta* plants in apical part for reacom actions increased on 28 % in comparison with the control. Reacom positive influence on development of rhizomes was kept even when oil was added to the soil – diameter of rhizomes was 24 % much with respect to the controls. Emistym did not influence on the growth of rhizomes, diameter remained within the limits of the control. Diameter of rhizomes was 20 % lower than in the controls under compatible action of emistym and oil. The complex application of reacom, emistym and oil had a positive influence – diameter was16% higher compared with the control and it did not differ significantly from the separate influence of reacom. Thus, for optimization of growth of sedge rhizomes in the conditions of oil contamination it was enough to use only reacom.

Level of chlorophyll sum (*a+b*) and their correlation in the leaves of *Carex hirta* plants under the action of oil contamination, reacom and emistym C were determined. The chlorophyll *b* content increased compared with the chlorophyll *a* content and that could be explained by adaptation of plastids of plants to the stress conditions. The decrease of green pigments concentration under complex influence of reacom, emistym and oil was observed. The content of chlorophyll did not differ significantly between controls and emistym C (dilution $1x10^3$) treated plants. Such results were obtaine in plants from oil polluted pots under the action of reacom. This confirms the inexpediency to connect reacom and emistym C for the treatment of plants which grow on oil contaminated soil.

PECULARITY OF HORMONAL REGULATION OF α-AMYLASE ISOENZYMES IN EMBRYO AND ALEURONE OF CEREAL GRAINS

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In cereal grains α-amylase carries out a key role in the starch mobilization during germination. Enzyme synthesis localized in two parts of grain, they are aleurone and scutellum, and then begin the process of its secretion to endosperm. The α-amylase synthesis induced whith phytohormone GA_3, another hormone ABA carries out the opposite activity on this process.

In sprouting cereal grains there are more then ten individual α-amylase izoenzymes, which divided on two basic groups according their isoelectric points and some other biochemical properties and physiological destination. In the given work it was investigated the influence of GA_3 and ABA on the synthesis induction of different α-amylase isoenzymes in aleurone and scutellum of cereal grains.

In the recent work used with and without embryo rice half seed (Oryza sativa L., variety Marzhan), and the same, soft wheat (Triticum aestivum L., variety Saratovskya 29) material. In the incubation mediums put 2 μM of phytohormones. After incubation during 1 up to 5 day in the isolated embryo and aleurone layers studied α-amylase activity and isoenzyme components.

Obtained data specify existance of the significant distinctions in α-amylase synthesis in embryo with scutellum and aleurone cells. Scutellum cells are poorly susceptible to the hormone signal, which is expressed in α-amylase activity change ability. In the case of embryo half seeds hormones addition did not lead to α-amylase activity change at least the first 72 hour of incubation. On the contrary deembryonated half seed showed high sensitivity to hormone action. GA_3 presence lead to sharp α-amylase activation already at the initial incubation stage, during 24-48 hours, ABA had braking effect on this process.

With the help of native electrophoresis was shown the different isoenzymes sensitivity on GA_3 and ABA action. On without embryo half seeds was shown that GA_3 induced the synthesis of anodic (group A) isoenzymes and repressed the activity of cathodic (group C) enzymes. Opposite action rendered for ABA at which presence synthesis of α-amylase group A was completely suppressed.

Summarising the above-stated and our research data it is possible to conclude that in cereal grains the synthesis of α-amylase isoform with low isoelectropoints (group A) is strictly adjusted by endohormones level (GA_3 and ABA). The mechanism of an high isoelectropoints (group C) possible is more combined and can include double control – phytohormones and sugarmetabolites action.

THE SILKPREPARATION EFFECT UPON CUCUMBER PLANT GROWTH IN HYPERTHERMAL CONDITIONS

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To minimize the influence of stress factors upon plants one may use certain growth regulators, of which cytokinin substances have been well studied. For the recent years, there have been synthesized new preparation whose effect is not thoroughly studied, for instance, the SILKpreparation which represents itself a water emulsion containing the ether extract (abietine acid as the acting substance) of the Sibirian fir tree – Abies sibirica Ldb.
The purpose of the present work is to study the SILKpreparation effect upon the cucumber plant growth within various temperature ranges. Seeds and plants of Cucumis sativus L of the "Iziaschny" sort are subject to our investigation. Previously selected cucumber seeds have undergone the SILKpreparation treatment in the concentrations 10-6 %-10-7 % during 8 hours. After the preseminal soaking, the seeds were grown by the method of soil culture. Upon the appearance of seed-lobe leaves in the sprouts, our experiment involved 2 temperature conditions: 22-23°C (control group), 35-36°C (increased temperature), the unfavourable temperature mode being imitated for 3 days. Upon the development of the third real leaf, the cucumber plant was placed into the open soil in the beginning of June. Upon the development of the 5-th real leaf, we have under taken an additional out-of-root treatment of the plants by their sprinkling with the SILKpreparation. For the plants treated by the SILK concentration 10-6 % we applied a higher 10-3 % concentration, whereas for the plants treated by the SILK concentration 10-7%, we applied the concentration 10-4%,
The experimental results showed the 10-7 % concentration to produce a stimulating effect on the height of the plant over-ground part at the optimal temperature. The estimates in this variant exceeded the control values by 33 %; a higher concentration in the optimal conditions being not effective. At an increased temperature, both concentrations produced a move pronounced stimulating effect. The use of the out-of-root treatment increased the area of leaf surface, as well as wet and dry mass of the over-ground part of the plants in all temperature variants. The highest wet mass augmentation, by 85 % as compared to the control variants, was observed in hyperthermal conditions. The root wet-mass at 22-23°C increased by 110 % and 142 %, as compared to the control group, at various concentrations. In the conditions of the increased temperature this augmentation reached greater values, i.e. 131 % and 156 %, respectively. All the concentrations under the experiment produced a stimulating effect on dry-mass augmentation at the optimal temperature. At in increased temperature, the stimulating effect upon this parameter was produced only by a much lower concentration in other words, the seed and plant treatment by the SILKpreparation is conducive to the watering of cells and tissue of the roots, thus stimulating the growth and in crease of the suction surface. The seed treatment by this preparation contributes to the growth of the total number of flowers on the sprouts, especially, at a high temperature, and changes the ratio of mal-female flowers in favour of the female ones. Much lower concentrations of the SILKpreparation increased the crop capacity at the expense of the augmentation in quantity and mass of cucumbers in all variants to an equal degree.
Thus, the cucumber seed preseminal treatment by the SILKpreparation followed by sprinkling of this preparation augmented the female flower sexualization, quantity of cucumbers from one plant, and mass of the vegetables. Lower concentrations of the SILKpreparation in the range 10-7 %-10-4 % turned out to be more effective, the crop capacity being greater at higher temperatures.

NEW REGULATORY COMPOUNDS FOR PLANT GROWTH AND RESISTANCE

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Modification of the hormonal balance is a strong factor of regulation of the production process under unfavorable conditions. Therefore treatment of plants with natural or synthetic regulators of growth is widely used to increase plant resistance. It was demonstrated that treatment of plant leaves with methanol, a low-molecular one-carbon alcohol, caused a decrease in the negative impact of water deficiency on the production process of plants. It should be noted that methanol is a toxic agent, which makes it impossible to use this alcohol for practical purposes. Like methanol, some of its nontoxic analogues (LLB, PNBA, Fpeg, Fty, etc.) were also shown to have a positive effect on plants. By now some of these agents have been patented and adopted for practical use. However major effects of these agents on the general metabolism of plants should be studied in experiments with methanol, because this compound is simpler and more closely associated with plants than its analogues. It is very important to use methanol in model experiments, because it is produced in plant leaves and play a possible role in the enhancement of plant resistance. The goal of this work was to study the rate of CO_2 gas exchange, transpiration, stomatal resistance, and efficiency of the use of water in sugar beet leaves of different strata treated with methanol solution and to assess the stability of the photosynthetic apparatus against the background of increasing water deficiency in soil. The activities of some enzymes were also estimated.

Sugar beet (*Beta vulgaris*) plants were grown in controlled conditions with a temperature regime 30/30 °C (day/night), relative air humidity 70%, and 24-h light/dark photoperiod with a light intensity 1000-1200 μmol m^{-2} s^{-1}. Measurements and methanol treatment were carried out using 40- to 65-day-old plants at the growth stage of 5–7 leaves. The plants were sprayed with a solution containing 40% methanol, 1 mM glycine (source of nitrogen) and 200 ppm detergent sylvet (pH 6.8). Control plants were treated with the same solution but without methanol. During the next 9 days of the experiment the volume of water used for watering each jar was gradually reduced. As a result the plants during the experiment were exposed to conditions with high intensity of light, enhanced temperature, and gradually increasing water deficiency in soil (from 0 to –100 kPa). The resistance to stress caused by water deficiency in soil was 1.3–1.9 times higher in the plants treated with methanol, than in the control plants. The methanol foliar spray eliminated the negative effect of water stress on photosynthesis, transpiration, and stomatal conductivity. Namely, under drought conditions, Rubisco activity, rate of CO_2 assimilation, stomatal conductivity and the rate of transpiration were higher in the methanol-treated plants as compare to control plants. Increase in malic enzyme activity indicating activation of anaplerotic way of carbon metabolism was also observed in the methanol-treated plants.

The obtained results show that under drought conditions methanol foliar spray induces stress-tolerance and photosynthetic productivity of sugar beet plants. Using of the nontoxic methanol analogues may promote defense of cultural plants from the climate aridization.

NEW APPROACH FOR PREDICTION OF BIOLOGICAL ACTIVITY OF NEW CHEMICAL COMPOUNDS BY ARTIFICIAL NEURAL NETWORKS

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The major goal of this research was to develop a robust QSAR-based expert system suitable for virtual screening of available libraries and known drug databases to identify compounds possessing new formerly unknown activity. To achieve that, we have proposed a new approach that combines Kohonen Self-Organising Map (SOM) and Associative Artificial Neural Networks (ASNN) for the analysis of quantitative structure-activity relationships (QSAR) in the absence of training set of compounds. This algorithm has been developed for the analysis of CoMFA (Comparative Molecular Field Analysis) series in 3D QSAR. CoMFA generates thousands of interaction energies based on a lattice of points surrounding the structures of the analyzed molecules. An analysis of such numbers of points is complicated with traditional Artificial Neural Networks due to high-dimensionality of the input data set. The SOM of Kohonen was used to discover similar regions of the 3D input datapoints while the ASNN method establishes relationships between these regions and biological properties of the analyzed molecules. The main difference of the proposed Volume Learning Algorithm (VLA) compared to the previous approaches is its ability to take into account spatial information of the input data set, to compress this information and to automatically determine the most informative regions of the input data.

At the first stage we have employed SOM of Kohonen in automated data classification. The SOM is often able to cluster compounds according to the mode of action or target of the drugs. At the second stage compounds divided on the classes were analysed by VLA using a leave-one-out cross-validation procedure. The importance of the detected clusters for the observed activity was evaluated using pruning methods during the last stage of the algorithm. The pruning eliminated a number of clusters detected by the VLA procedure. Clusters with the largest number of parameters were detected as non-significant and were eliminated by the pruning algorithms. The quality of received models was confirmed by experimental spot check of predicted activity of compounds. It was shown that proposed approach allows effectively reveal compounds possessing new formerly unknown activity and can be used for computational screening for new drugs development.

PHYSIOLOGICAL EFFECTS OF ACTION ULTRA-VIOLET RADIATION ON REGENERANTS OF POTATO.

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Naturally plants during all life cycle influenced various factors of an environment. Special value ultra-violet radiation (UVR) (has 180-400 nanometers), a part of electromagnetic radiation of the Sun. According to forecasts, the future global changes of a climate connected with an exhaustion of an ozone cloud, entail increase in a doze of radiation getting on Earth UV. In this connection the knowledge of mechanisms of action UVR on physiological processes at plants, and especially on agricultural crops, gets the big theoretical and practical value. The primary goal of our research - to establish physiological effects of action UVR on growth, development and biological efficiency of a potato. Researches are executed on regenerants of potato (Solanum tuberosum L.) grades Odyssey and Yavor the Belarus selection, which grew up under lamps DNAZ-400 (the photoperiod – 16 hours) on artificial substrata at a room temperature. As source UVR mercury lamp DRT – 1000 served. For the control of size of a doze of an irradiation of plants used UVR – dosimeter DAU – 81. The unitary doze (E1) regenerants of potato made UV-irradiations 120 Dg/m^2. Variants of experience carried out all in 3-5 multiple frequencies.

During experiment it is established, that action UVR on plants of a potato causes the certain changes in the general metabolism and physiological reactions regenerants. At UV an irradiation stimulation growth processes, increase in the contents of a chlorophyll *a* and *b*, *car*, flavonoids in leaves regenerants was observed. Irradiation UVR stimulated formation and development of roots, and rooting control regenerants on the average occurred for the seventh day after grafting, and regenerants irradiated UVR took roots on the third - the fourth day. The irradiated plants had higher factor of duplication (on 25-33 %) in comparison with the control that is very important parameter for primary seed-growing a potato. The contents of dry substance in the tubers received from UV-irradiated regenerants, was authentic on 11 % above in comparison with the control. Researches on revealing influence UVR on physiological processes of the plants which have been grown up in artificial conditions, allow using UVR for stimulation and the directed synthesis of organic substances in plants, to change duration of physiological phases of their development. The opportunity of application UVR from artificial light sources in vegetative constructions and controllable conditions for cultivation of the improved landing material of vegetable cultures will allow increasing their productivity and quality.

IMPACT OF CHOLINE-CONTAINING COMPOUNDS ON GROWTH, GREENING AND PHYTOHORMONE BALANCE

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Known growth retardant - 2-chloroethyl-trimethylammonium chloride (CCC) and its analogue 2-ethyl-trimethylammonium chloride (CC) were shown to have influence on growth and greening of etiolated wheat seedlings. It is known that changes in balance of phytohormones play an important role in effects of growth retardants on plant growth and activity of the photosynthetic apparatus (PA). The aim of our research was to study the interrelationship between changes in levels of hormones and effects of choline compounds on growth and greening of wheat and bean seedlings. Photochemical activity of PS II was assessed by measurements of chlorophyll *a* variable and delayed fluorescence using phosphoroscope. The pigment contents were determined spectrophotometrically. The levels of abscisic acid (ABA) and cytokinins were assessed by enzyme-linked immunosorbent assay. The effects of CCC and CC at concentrations of 1 μM –5mM on growth, greening and formation of the PA in etiolated wheat seedlings were examined (1). A short-term root application of CC and CCC inhibited elongation of the coleoptile and first leaf but cholines accelerated these growth responses when the seedlings were exposed to white light. The first leaf appearance was accelerated as well. Effects of cholines were observed during 96 h of light exposure after the pre-treatment of 4-d-old seedlings with cholines and depended on the type and concentration of compound. CCC and CC accelerated greening and increased the photochemical activity of PS II in seedlings. Stimulation of greening by cholines was accompanied by accelerated accumulation of cytokinin-like substances (2) detected in the first leaves. Taking into account that root application of kinetin (10^{-4}-10^{-6} M) stimulated greening we concluded that the influence of cholines on concentration of substances with cytokinin activity detected in leaves might be involved in the stimulation of Chl (*a*+*b*) accumulation. Root application of GA_3 (10^{-4}-10^{-6} M) lead to partial decline in inhibitory effect of CCC on leaf and coleoptile growth. Besides, pretreatment with CCC led to increase in level of ABA and decline in content of gibberellins in primary leaves of bean (*Phaseolus vulgaris* L.) seedlings. Thus, inhibitory effects of choline compounds on growth may be due to enhanced formation of ABA and reduced level of gibberellins resulting in choline pretreatment.

References

1. Kobzar', E. F., Kreslavski, V.D., Kuznetsov, E.D., Muzafarov E.N. Photosynthetica 1999. 36: 333-340.
2. Kreslavski V., Kobzar' E., Ivanova E., Kuznetsov E. Plant Growth. Reg. 2005. 47: 9-15.

STIMULATING EFFECT OF THE SUPERSLOW DOSES OF THE MIXTURE OF ORGANIC ACIDS TO ACCLIMATIZATION CUTTINGS OF GRAPES

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At present the growth regulators in the viticulture in essence adapt with the production of the inculcated and root landing material for purposes of stimulation root -, callus formation and the coalescence of implanted components.

The following re growth gulators are most common in practice: heteroauxin or β-indolilacetic acid (IUK), potassium salt of heteroauxin, indolylbutyric acid (IMK), α-naphthylacetic acid (NUK) and its potassium salt (KNUK). The new growth regulators are: epin, nikfan, ale-1, kornevin, Caucasus, universal, ekzuberon and others.

The purpose of this work is to study the influence of a number of low-molecular carboxylic acids at the superslow doses on acclimatization of the grape cuttings.

The following organic acids were used as the growth stimulators : citric $CH_2C(OH)CH_2(COOH)_3$, α-ketoglutaric $HOOCCOCH_2CH_2COOH$, succinic $C_2H_4(COOH)_2$, malic $HOOCC(OH)CH_2COOH$, oxalic $(COOH)_2$. The solutions with different molar relationship of acids were prepared, namely, solution №1 (1:1:1:1:1), №2 (1:2:3:4:5), №3 (5:4:3:2:1).

The following types were studied: Katyr-2, Muscat Katunskiy. The concentrations of solutions №1, №2, №3, succinic acid (SA) were 10^{-11} M. The succinic acid, heteroauxin and epin were used as the standard. Control was water. Cuttings were immersed in the solutions on deep of 4 cm, solutions changed every 4 days. The duration of experiment was 40 days. Number of cuttings was 10 in each experiment. The cuttings, which did not give roots, were not considered.

The conducted investigations were showed that the output and the quality of cuttings depends both the used growth stimulators and on quality special features. Thus, in type Muscat Katunskiy the experienced preparations were showed no positive effect on the output of cuttings, it proved to be lower than in the control.

However, stimulators had positive influence on the development of shoots and root system. For the type Muscat Katunskiy the best results on the development of shoots were observed with the processing by solutions №1 and №3 (addition relative to control by 162%, 217% respectively); to the development of root system to the greatest degree contributed solutions №3 and SA (addition relative to control in terms of a quantity of roots for the solution №3 179% and along the length of roots for SA 134%).

The influence of the mixture of organic acids on output and quality of cuttings of the types of Katyr-2 was more essential. Thus, the output increased relative to control by 133%, 133%, 200% the processing of the type Katyr-2 by solutions №1, №2, №3 respectively. However, shoots were developed better in the control. The highest results in quantity and along length of roots were observed in the version with processing SA (addition relative to control in terms of a quantity of roots of 195% and along the length of roots of 165%).

Thus, it was established the best result of the carried out experiment that processing grape cuttings by solutions №2, №3 and SA has a positive effect on acclimatization of cuttings, which in the final analysis increases output and quality of cuttings.

SECONDARY HORMONE OF CYTOKININ AND 14-3-3 PROTEINS ACTIVATE Ca^{2+}-DEPENDENT ATP-ASE OF PLASMATIC MEMBRANE FROM ALEURON LAYER OF WHEAT SEEDS

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For the first time fusicoccin like cytokinin secondary hormone (CSH) was discovered in our laboratory.

It is well known that fusicoccin activates H^+ATP-ase of plasmatic membrane. In this reason, it was necessary to test the effect of CSH on the activity of H^+ATP-ase of plasmatic membrane from aleurone layer of wheat seeds.

Unfortunately CSH doesn't activate H^+ATP-ase of plasmatic membrane which was isolated from unembryonated wheat seeds. When CSH acts on whole seeds an effect of activation take place.

From this experiment it is possible to make next important conclusion. The CSH causes the formation of unknown regulatory factor in the embryos of wheat seeds.

It is well known that fusicoccin works only with 14-3-3 proteins. In this reason we assumed that CSH causes the formation of 14-3-3 proteins in the wheat embryos. And then formed 14-3-3 proteins are translocated to the aleuron layer of wheat seeds.

To check this hypothesis we carried out the next experiment. It was taken the embryo parts of wheat seeds and they were soaked by CSH solution 0.23 mkg per ml.

After 2 hours of soaking the embryo parts of wheat seeds were homogenized, and then the cell-free extract was used. Also it is well known that brain contains huge quantity of 14-3-3 proteins. We also received the cell-free extract from sheep brain.

For the experiment unembryonated wheat seeds were soaked on 3 hours in CSH and the cell-free extracts from wheat embryos and CSH and cell-free extracts from sheep brain. Both extracts strong activate ATP-ase of plasmatic membrane from unembryonated wheat seeds.

The investigation of properties of activated ATP-ase of plasmatic membrane of aleuron layer shows that this enzyme related to Ca^{2+} dependent ATP-ase.

Namely this Ca- ATP-ase participates in increasing of the level of cytosolic Ca^{2+} in the cells of higher plants. It was very surprisingly that CSH and cell free extracts from wheat embryos and CSH and cell-free extracts from sheep brain causes the activation of ATP-ase of plasmatic membrane of aleurone layer only with ions but not with Mg^{2+} ions.

ATPUM4, A MEMBER OF PUF-DOMAIN RNA-BINDING PROTEINS, IS ESSENTIAL FOR FEMALE GAMETOGENESIS AND EARLY EMBRYO DEVELOPMENT

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Pumilio, a RNA-binding protein (RBP) that has a typical feature of eight tandem repeat domains (PUF domains) comprised of about 35-39 amino acid residues, has been known to repress translation activity especially. Since the first report on its binding propensity to maternal hunchback mRNA localized to posterior pole in fruit fly, Pumilio has been characterized in many eukaryotes including yeast, nematode, and vertebrates including human. This RBP was found in plant as well, whereby Arabidopsis and rice possess 23 and 15 genes encoding PUF domain proteins that are in turn categorized into 3 groups. Here, we report a Pumilio gene (*AtPUM4*) that is potentially involved in nucleolar functions in Arabidopsis. AtPUM4 is expressed in all the organs tested, and upregulated in the presence of glucose. Null mutant was not able to produce fertile seeds where zygotic embryo was arrested in globular stage, while 35S::AtPUM4 plants didn't show any developmental changes under normal growth condition. Besides embryo development, AtPUM was involved in female gametogenesis as evident from the result obtained reciprocal cross between AtPUM4 heterozygote and wild-type. AtPUM4::GFP fusion was mainly localized to nucleolus with a background level of distribution in nucleoplasm, which suggests the roles of AtPUM4 on the processing of RNAs resident in ribosome. As expected, 5'ETS of pre-rRNA was not effectively processed in the inducible RNAi transgenics, suggesting the involvement of AtPUM4 in U3 snoRNA metabolism.

USING OF ETHYLENE AND FATTY ACIDS AS MARKERS OF WHEAT VARIETIES

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Different varieties of winter wheat possess in reasonable levels of growth regulating substances that provide their growth, productivity and resistance to biotic and abiotic stresses. Nevertheless, the role of natural plant growth regulators in the growth and development of each variety is not elucidated. New methods to be used in the selection and variety certification are presented in this report. We have found that winter wheat leaves of different (resistance to fungi and lodging) varieties produce different amount of ethylene (Table 1).

Table 1. Synthesis of ethylene by leaves of 2-week-old winter wheat plants.

Varieties	Date of ethylene detection							
	22.02.05		14.03.05		28.03.05		13.06.05	
	Height of plants, cm	Ethylene nL/g FW	Height of plants, cm	Ethylene nL/g FW	Height of plants, cm	Ethylene nL/g FW	Height of plants, cm	Ethylene nL/g FW
Columbia	14,2±0,15	150±7	12,4±0,22	220±11	13,4±0,41	180±8	14,3±0,14	130±6
Smuglianka	15,1±0,15	160±11	14,0±0,33	200±22	14,1±0,32	200±19	-	-
Yatran 60	17,2±0,28	85±8	14,4±0,38	200±16	14,8±0,64	60±6	16,4±0,31	84±6
Kyivska 8	14,3±0,16	100±6	14,1±0,23	80±5	14,7±0,29	50±3	15,4±0,42	54±4
Mironivska 808	-	-	18,5±0,74	45±5	18,3±0,68	45±3	17,9±0,72	38±3
Mironivska 61	22,3±0,19	16±4	17,6±0,83	40±4	19,2±0,72	36±6	20,2±0,55	39±4
Podolianka	20,2±0,22	13±5	14,8±0,69	35±4	16,5±0,69	40±5	16,3±0,54	15±3
Bilotserkivska semi-dwarf	15,2±0,16	15±2	14,8±0,44	40±4	14,4±0,31	35±3	16,5±0,31	23±3

Another method that characterizes any plant is fatty acid composition of coleoptiles (Table 2). Experiments were performed when 1st leaves were1-5 mm higher than coleoptiles.

Table 2. Fatty acids content in the winter wheat coleoptiles.

Varieties	Height of coleop-tiles, mm	Fatty acids content, μg/g FW								
		C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C24:0	Total content
Columbia	40,1±1,2	178±8	7,3±0,27	16,4±0,7	213±9	96±4	2,4	3,0	0,5	516,6
Smuglianka	45,2±2,1	181±11	7,6±0,33	16,7±1,2	217±14	86±9	2,2	3,0	0,7	514,2
Perlyna lisostepu	45,1±2,2	174±7	6,9±0,26	15,8±0,6	209±8	89±5	2,1	3,0	0,5	500,5
Mironivska 808	48,0±2,0	163±12	7,7±0,35	16,8±1,2	197±13	81±8	2,9	3,0	0,5	471,9
Mironivska 65	57,3±2,3	162±7	7,2±0,38	16,0±0,9	177±9	84±7	2,3	2,4	0,4	451,3
Podolianka	58,2±2,4	142±6	8,6±0,39	14,5±0,7	160±7	74±4	0,5	1,0	0,5	401,1
Mironivska 61	55,3±2,2	119±4	5,6±0,26	9,4±0,5	143±5	70±4	1,0	1,6	0,5	350,1
Bilotserkivska semi-dwarf	50,6±2,0	89±5	7,6±0,22	6,9±0,5	123±4	58±4	0,3	0,5	0,3	285,6

We believe that presented here data may be used in the selection of new varieties with required traits and as variety markers of plants.

EFFECT OF SYNTHETIC PREPARATIONS ON PEROXIDATION PROCESSES IN CORN ROOTS UNDER SALINITY

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Salinity is one of the main abiotic factor that decreases the plant production via the osmotic and ionic balance disturbance and intensifying of the peroxidation processes. The effect of synthetic preparations Methyure and Ivine on level of enzymatic and non-enzymatic peroxide oxidation in corn seedling roots have been studied.

Corn seedlings (hybrid Kolektyvnyi 225 MV) were grown on Hoagland medium during 7 days and then exposed to fresh nutrient solution contained 0.05 and 0.1 M NaCl during 1 and 10 days. Synthetic compounds were used by seed soaking in their 10^{-7} M water solutions. Intensity of non-enzymatic peroxidation processes was measured by H_2O_2–induced chemiluminescence (ICL) technique. The LOX activity was measured spectrophotometrically at 234 nm.

One-day exposition of corn seedlings to 0.05 M NaCl increased ICL level in root homogenates, whereas the 10-day ones didn't change the intensity of non-enzymatic peroxidation. On the contrary, seedling exposition to 0.1 M NaCl during 1 and 10 days increased ICL intensity significantly in root homogenates. One-day exposition of corn seedlings to 0.05 M NaCl increased LOX activity, whereas the 10-day ones had contrast effect. The seedling exposition to 0.1 M NaCl during 1 and 10 days decreased LOX activity. Seed treatment by both synthetic preparations decreased oxidation level and stabilized LOX activity in root homogenates of salt-stressed seedlings.

Using of synthetic preparations decreased the intensity of peroxidation processes in corn roots under the salt stress conditions and stabilized LOX activity. Therefore, these compounds can intensify the salt tolerance of corn seedlings.

PHYTOHORMONAL OPTIMIZATION OF NUTRIENT MEDIUMS FOR THE EFFECTIVE CALLUS INDUCTION FROM SEEDS AND TISSUES OF THE HERBS

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The cultures of isolated tissues and cells are widely used as secondary metabolites producers for the medicine, perfumery, cosmetology and other branches of industry.

As a advantage of method preparation of secondary metabolites - is in the possibility of obtaining of the production whole–yearly, as well as the possibility use the herbs, non-growing in our natural conditions.

Competent callus of herbs have been obtained for the purpose of studying of secondary metabolites. We investigated influence of hormones on callus formation and selected concentrations and correlation hormones in medium for callus culture and suspension cultivation.

Callus could be induced from seeds on standard Murashige and Skoog medium. The obtained explants were divided on organs and planted on phytohormones containing medium. Cultivation conditions: temperature 26±1 ^{0} C, darkness, for 30 days.

It has been established that MS medium with the addition of auxin 2,4-dichlorophenoxyacetic acid (2,4-D) in concentration 2mg/l and saccharose concentration 3% is the most optimal for the stimulation of callus formation of Rubus idaeus L., Rubus caesius L, Melissa officinalis. An improvement of auxines action due to indole-3-acetic acid (IAA) addition in concentration 0,25mg/l alpha-naphthalenacetic acid (NAA) in concentration 0,25mg/l, and cytokinin of kinetin in concentration 1 mg/l was found to be optimal for the subcultivation. The best result for the callus formation for Menta piperita was obtained on the medium with 0,2mg/l 2,4-D in combination with NAA - 0,4mg/l and 6-benzylaminopurine (BAP) - 1mg/l. Embryogenic callus with numerous spherical somatic embryos could be induced from wound surface of stalk explants Salvia officinalis cultivated on the medium with cytokinin tidiazyrone (TDZ) in concentration 5mg/l, 3mg/l and 1mg/l. A high percentage of proliferated callus was obtained on the medium containing 1mg/l TDZ. The initiation of callus obtaining from Digitalis purpurea L. was stimulated on the basic MS medium containing increased concentration 2,4 D-5mg/l. We have investigated the frequency of callus Aloe arborescens formation using apical part, medium and inferior part of the stalk. The optimal dedifferentiation was observed in the tissue of young stalk on the medium containing NAA 25mg/l and kinetin in concentration 0,05-0,1mg/l.

The data obtained are planned to be used for biotechnological purposes.

BIOPREPARATION "MYCOLIN" AND ITS EFFECT ON BARLEY PLANTS

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Plant treatment with different physiologically active compounds, as a chemical method for regulating productivity and resistance, is successfully applied for a long time. In spite of its high efficiency and economical expedience, the necessity for environment conservation requires more extensive application of biological methods. In this connection development and introduction of adaptive forms of plant-growing, based on use of biological communities emerging under plant-microbe interactions, become rather urgent. Employment of substances of a biotic origin and application of biopreparations based on living microorganism cultures are proposed. Microorganisms developing in a host-plant complex over the whole period of vegetation can regulate plant growth, development and productivity affecting different physiological processes. On the other hand, such preparations can improve even immune properties of plants inducing changes in metabolism of a susceptible host to a side unfavorable for a parasite. They can exert a mediated effect on plant productivity being involved in defense reactions of plant and increasing its resistance.

The effect of the biopreparation "Mycolin" developed in the laboratory of mycology at the Institute of Experimental Botany of the National Academy of Sciences of Belarus, on sowing qualities of seeds, disease resistance and productivity of barley in different cultivars was studied in laboratory and field trials.

Seed steeping in preparation solutions for 24 h was shown to exert a positive effect on germination and initial growth of barley seedlings. Germination energy increased by 8-16% and germinating capacity did up to 6% depending on a cultivar and mycolin concentration. A stimulating effect of the biopreparation on seedling growth was observed. Subsequent field trials have shown that mycolin did not exert a substantial effect on stalk growth and terms of basic developmental stages. However, preparation treatment (seed steeping in solutions and plant spraying along vegetative mass) changed productivity and structure of yield as well as favored reduction in the developmental rate of barley leaf diseases. Gains in grain yield were primarily achieved owing to such structural productivity elements as productive tillering and increased 1000-grain weight that probably points to the presence of a regulatory effect of the preparation.

For finding out the physiological role of mycolin in regulating disease resistance and productivity of barley plants, the state of a photosynthetic apparatus was studied for the pigment content in leaves as well as the state of membranes was estimated by the content of lipid peroxidation products. Such parameters are important for assessment of the functional plant state when there are no visible changes. At the same time a stimulating or an inhibiting effect of the preparation can be judged by the change in the pattern and direction of metabolism. Mycolin was shown to stimulate photosynthesis processes increasing the content of photosynthetic pigments in leaves and stabilizing the state of membrane systems in plant cell.

NMR & LC-MS STUDIES OF BIOACTIVE COMPOUNDS PRODUCED BY ENDOPHYTIC *METHYLOBACTERIUM* OF SCOTS PINE

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Internal plant microbes, endophytes, have during the recent years become in the spotlight of research. Endophytes are essential for plant defense, and numerous reports point to action of endophytes in plant growth and development. The *Methylobacterium* endophytes affect morphology and extend viability of Scots pine tissues through bioactive products. Infection of pine seedlings by the *Methylobacterium* endophytes increases lateral root formation, root length, and biomass. The biological significance of the *Methylobacterium* endophytes is mainly unknown

The compounds produced by endophytic *Methylobacterium* were studied using HPLC-TOFMS, HPLC-MS/MS and NMR. Isolation and purification of compounds were done by flash chromatography and preparative high performance liquid chromatography.

REGULATION OF FLOWERING TRANSITION OF *ARABIDOPSIS THALIANA* MUTANTS BY GIBBERELLIN

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The current research into the regulation of plant transition from the vegetative to reproductive development is focused on progressive characterization of the genes participating in the control networks and clarification of the gene interactions and their relation to external inducers.

Presently the major ways of interaction of *Arabidopsis thaliana* genes for transition to flowering are disclosed. Crucial genes of such pathways are induced by external inducers (photoperiod, temperature and gibberellin). According to the modern molecular- genetic concepts, gibberellin accelerates the initiarion of flowering of *A.thalliana* plants by activating gene LEAFY.

We aimed to discover genetic and hormonal regularities of transition regulation of Arabidopsis plant from vegetative to reproductive morphogenesis that gives an opportunity to find out the role of gibberellin in flowering regulation.

We studied *Arabidopsis thaliana* long-day plants with the quantitative-type response to photoperiodic induction, plants had CONSTANS gene mutation. CONSTANS is the gene, which participates in florigen formation in leaves under photoperiod induction. Mutants were derived from the line Landsberg erecta – mutant №176,179, and line Columbia – mutant № 3325, 3122. seeds were received from the Nottingham Arabidopsis Stock Center. Mutants exhibited various delay of flowering.

Plants were grown in the chambers illuminated by cool white fluorescent lamps (LB 80) at 22-24ºC in long (16h of light and 8h of dark) and short days (8h of light and 16h of dark), at 80% humidity. Plants were sprayed with gibberellic acid, at 300 mg/l. Measurements (plants height, length, width of leaves; length of flower stalk; date of flower buds appearance) were executed twice a week.

We compared the rate of flower bud appearance in wild type (WT) *A.thalliana* plants on short and long days and mutants on short day. Most effectively gibberellin accelerated flowering of WT plants, the acceleration was 41 day. Acceleration of flowering in mutants № 3122, 3325 was 17 and 30 days, respectively. Mutant 176 had acceleration of flowering equal to 11 days. However, gibberellin did not influence on flowering initiation in mutant №179.

To conclude, gibberellin influences flowering of CONSTANS-mutants. The degree of acceleration depends mutation specificity. The difference between the rates of flowering initiation after gibberellin treatment probably indicates that during transition to reproductive morphogenesis gibberellin not only acts on the expression of gene LEAFY localized at the shoot apex, but also has relation to a gene network of photoperiodic regulation of flowering, where one of the most important genes is CONSTANS.

THE MECHANISM OF PATHOGENESIS INDUCED BY HERBICIDES INHIBITORS OF ACETYL-CoA CARBOXYLASE

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The mechanism of patogenesis induced by herbicides inhibitors of acetyl-CoA carboxylase (ACC) was investigated. Effect of herbicide haloxyfop-R-metyl (HRM) on lipids peroxidation (POL) reactions and on dynamics of necrosis induction in meristematic regions of maize roots was studied. The increase of POL reactions related to HRM action was not observed in integral lipids of meristematic cells, but lipoxygenase inhibitor – 2,4-D and free radical scavenger – tocopherol retarded the appearance of necrosis induced by HRM. The conclusion has been done that POL reactions do not be the direct reason of cells death induced by HRM in maize root meristems. The possible role of reactive oxygen species, plant signal systems and programmed cell death in mechanism of pathogenesis induced by ACC-inhibiting herbicides has been discussed.

EVALUATION OF ANTIOXIDANT ACTIVITY OF 3-SUBSTITUTED 5,7-DIHYDROXY-4-METHYLCOUMARINS

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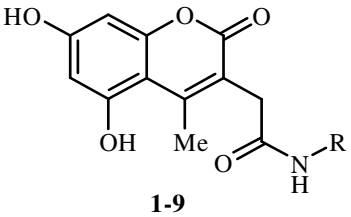
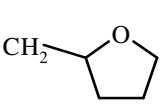
Natural coumarins and their synthetic structural analogs possess a broad spectrum of biological activity. The design of new derivatives of benzopyran-2-one is often based on research of the functions of these compounds in model systems. Coumarin derivatives containing meta dihydroxyls are definitely interesting for constructing potential synthetic antioxidants. Because of the importance of the 5,7-dihydroxy-4-methylcoumarin moiety in the structure of a potential antioxidant, we attempted to analyze certain properties of 5,7-dihydroxy-4-methylcoumarins containing 3-substituents. A series of structurally similar amides **1-9** was synthesized from 5,7-dihydroxy-4-methylcoumarin-3-ylacetic acid by the activated ester method using N-hydroxysuccinimide and diisopropylcarbodiimide or by reaction with N,N-carbonylimidazole. The reactivity of 3-substituted 5,7-dihydroxy-4-methylcoumarins **1-9** toward free stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical was analyzed [1].



1-9

R= $CH_2CH(CH_3)_2$ (**1**), $CH_2CH_2CH(CH_3)_2$ (**2**),



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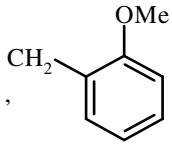

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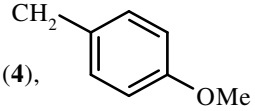

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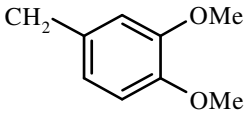

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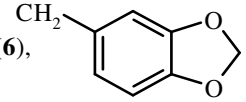

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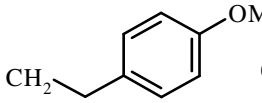

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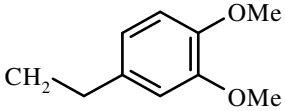

(**9**)

The results indicated that reactivity of 5,7-dihydroxy-4-methylcoumarin-3-ylacetic acid toward DPPH was unchanged compared with 5,7-dihydroxy-4-methylcoumarin. However, it about doubled on going to amides **1**-**9**. The nature of the amide fragment had little effect on the manifestation of their antiradical activity.

The effects of the synthesized compounds on xanthine oxidase activity and their antioxidative properties by scavenging the superoxide radicals were also found. Introducing a 3,4-dimethoxybenzyl or 3,4-dimethoxyphenylethyl substituent into the antioxidant structure (compounds **6** and **9**) markedly increases the inhibitory activity toward xanthine oxidase and effectively decreases the observed rate of superoxide dependent reduction of ferricytochrome C.

[1] O. V. Muzychka, M. M. Garazd, A. I. Vovk, I. V. Nagorichna, A. S. Ogorodniichuk. *Chem. Natural Compounds,* 2007, **43**, No 1, P. 19-23.

HORMONAL REGULATION OF DAY-NEUTRAL TOBACCO (DNT) PLANTS EFFLORESCENCE

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Flowering of photoperiodically neutral plants is related to the phenomenon of physiological gradient of flowering, I.e. successive efflorescence on the axis of main stem. . Research of hormonal balance of the DNT plants Trapezond at the direct and reverse gradients of flowering was conducted. By using stem girdling, a reverse flowering gradient was obtained in the tobacco plants. To study the distribution of phytohormones along the stem of tobacco possessing direct or reverse flowering gradients, the activities of endogenous gibberellins, contents of cytokinins and abscisic acid were estimated in bark tissues harvested from apical, middle and basal stem segments of flowering plants. It was demonstrated that at the normal, direct flowering gradient the activity of gibberellins and content of cytokinins in upper part of the stem was high, whereas the content of ABA was low. At the reverse gradient the mentioned relationship of phytohormones was characteristic of the lower stem part. The stem bark directly adjacent to axillary leaf bud, wherefrom the flower-bearing stem is actively developed, is characterized by a higher content of the phytohormones of the stimulator type and by a lower content of the inhibitor type hormones. Using HPLC on the C18 column for cytokinins analysis were shown that the formation of a flower-bearing state in the bark of DNT plants Trapezond is correlated with a sharp increase of the cytokinins level, especially isopentenyladenine and benzylaminopurine. Similar changes in the cytokinins level are shown under the direct and reverse flowering gradient. We made an attempt to displace the flowering gradient by the leave treatment by cytokinins on different stem nodes. The treatment of tobacco leaves (upper one at the reverse flowering gradient and lower one at the direct one) by the solutions of cytokinins led to the intensive stem growth and flowering. Findings specify on the substantial role of cytokinins in efflorescence of photoperiodically neutral plants.

STIMULATORS AND INHIBITORS OF GROWTH OF ALDER SPECIES UNDER PERMAFROST CONDITIONS

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We did not find the description of specific observation on root nodules (actinorhiza) growth in *Duschekia fruticosa* and *Alnus hirsuta* in the published literature under the conditions of the cryolithic zone of Sibiria. It should be noted that the taxonomy of this family is still disputable. Many researchers do not always distinguish a separate *Duschekia* genus from the initial *Alnus*. Besides, there is discrepant data on the effect of these plants enlargening on the growth and development of the conifers and other species. *Duschekia* overgrowing is an obstacle for self-seeding in conifers and its sprouting from the litter and roots inhibit much seed germination of the other plants. Recently while studying *D. fruticosa* buds in the pre-winter period we specifically isolated substances of stilbene origin pinocylvin and its methyl ether acting as a strong inhibitor in small concentrations over the growth and development of plants. So it is likely that pinocylvin from the leaf litter and subsurface organs of this plant can also affect allelopathically over the development of conifers and other species by inhibiting their growth.

Plants of *D. fruticosa* and *A. hirsuta* were studied during the summer-autumn seasons of 2006 in the vicinity of Yakutsk (62^0 N, 129^0 E). Each examined plant of both species had nodulation of a coral-like shape. We found to 25 g of nodules in dry weight near trunk area in the ring of 0.5 m in diameter. The root system was removed from soil and rinsed, nodules removed either, then dried and weighed. The ether fraction of the examined organs of plants was isolated by applying a thin layer chromatography method – isopropanol-ammonia-water solvent (10:1:1) – with further subdivision into ten zones to determine biological activity in each zone by means of biotesting for the gain of segments of wheat coleoptiles. 15% of stimulating or inhibition was taken as a confidence level. We did not determine nitrogen-fixing activity of nodules but it has been known that it is commonly in direct proportion to their amount and weight.

At bud and nodule testing of the plants studied for stimulators and inhibitors growth we found the following. There was no sure amount of inhibitors in *A. hirsuta* buds unlike *D. fruticosa* including those identified in R_f 0.9 zone linked with pinocylvin and its methyl ether. To the contrary, the buds of this plant have some zones (R_f 0.3 and 0.4) with a sufficient stimulating effect, probably, of the auxin origin. These differences between *D. fruticosa* and *A. hirsuta* by their set of inhibitors and stimulators in dormant buds can have a taxonomic value. This prevents the possibility of direct participation of actinorhizal nodules in producing biologically active substances with growth-inhibiting properties and their further transport into buds. Simultaneously, sure stimulating effect of almost all zones from nodulations of both species (except some inhibition in R_f 0.1 zone in *A. hirsuta*) was found while a pinocylvin fraction was not identified.

The growth inhibitors of the stilbene nature were not found in dormant buds of *A. hirsuta* unlike *D. fruticosa*. This gives the priority from biochemical angle to support the idea on identification of *Duschekia* genus from the *Alnus* one within the *Betulaceae* family. In nodules of both plants we did not find a reliable amount of growth inhibitors. Thus, it excludes the availability of significant synthesis of pinocylvin and its methyl ether in these structures. Simultaneously, the availability of a significant amount of stimulators in nodulations is recorded including those of the auxin origin, possibly used by microorganisms to maintain their efficient symbiosis with plants. Application of these alder species as N-fixing phytomeliorants should be provided at reclamation of sandy and damaged lands.

GROWTH AND DEVELOPMENT OF PLANTS UNDER THE ACTION OF DIFFERENT FRACTIONS OF SAPROPEL HUMATES

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Humic preparations are increasingly applied as stimulators in plant breeding. Humates of brown coal, peat and soils are mostly studied while sapropel humates (bottom organic sediments) are much less known. They are distinguished with abundance of highly molecular aliphatic structures sometimes called "sapropel" acids. The rate of biological activity of some fractions of preparations from sapropels of Yakutia which have been formed in specific conditions of permafrost are of particular interest.

The preparation has been made of frozen sapropel taken from Bolshaya Chabyda lake (near Yakutsk) by means of the alkaline hydrolysis (0.2 n NaOH) at heating with further centrifugal separation of sodium humates from sapropel mass. Three lyophilized fractions (1-3) (up to 50mg each) have been separated and accumulated by the method of column gel-chromatography on Sephadex G-100 and Sephacryl-300 (eluent-distilled water with pH 7.0; elution rate 10 ml/h) from the total preparation. They had curves of absorption specific for humates in UV-, visible and IR-area of the spectrum and molecular mass ranging from10 000 to 100 000 D. Middle (2)-and highly molecular (1), in particular, fractions were larger in volume unlike a low molecular one (3).

To examine their biological activity 0.0001; 0.001 and 0.01% concentrations have been used that is within the main stimulating range of the total preparation. Duckweed (*Lemna minor* L.), a water plant, has been taken as a test-object. The duckweed is specified with a rapid growth, simple structure and easily forms genetically homogenous clones. Additionally, the ponds with duckweed possess humic substances of the bottom sediments. The clone has been isolated from the local lake populations and maintained on 0.5 n Helriegel's or Hoagland-Snyder's mediums at the artificial lighting. Three fronds (mother's and two non-separate daughter's plants) of approximately the same size have been chosen for the experiment. The glass volume held 40 ml, 3-fold replica. After 12 days the plants were taken out, dried on the filter paper and green mass, number of plants and their outward appearance was defined. The main index was the change of time of the duckweed number needed for doubling in the studied solutions to the control (distilled water) expressed in the percentage Dt (%) = (1-(ln(Nc)-ln(N)/(ln(Nt)-ln(N))100%, where N is the initial number of plants, Nc – number of plants in the control, Nt – number of plants in the test at the same duration of observations.

All fractions showed a significant stimulating effect (but the 1st highly molecular fraction at 0.0001 и 0.001%). Green mass for fraction 1 (at 0.01%) was 36 mg (for the replicas) and averaged 26-54 mg for fraction 2 and 31-53 mg for fraction 3 (with all concentrations). Mean mass for the control amounted only 18 mg by the end of the test. Dt varied simultaneously and has made up: for fraction 1 (0.0001; 0.001 and 0.01%, respectively) – -3.4; 15.0 and 24.5% of doubling excess of the plant population unlike the control; for fraction 2 – 15.0; 13.0 and 40.6%; for fraction 3 – 25.8; 40.6 and 31.2%. So, the test-object *L. minor* shows a higher biological activity for middle (2) and particularly, low molecular (3) fractions of sodium humates in frozen sapropels at different concentrations as compared to a highly molecular fraction (1). These differences in the stimulating effect may be conditioned by a larger number of functionally active groups in fractions 2 and 3, and by their more powerful ability to be bound with cell receptors responsible for the growth processes, respectively.

The regularities found can be applied for searching physical-chemical methods and technologies relating to getting more highly effective and ecologically pure sapropel-based biopreparations and other caustobiolites.

STUDY OF INFLUENCE OF SODIUM AND POTASSIUM SAPROPELIC PREPARATIONS ON GROWTH AND DEVELOPMENT OF SPRING WHEAT IN THE CONDITIONS OF CENTRAL YAKUTIA

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Yakutia is one of the coldest regions of the world, where industrial cultivation of grain and vegetable cultures is still possible. It is obvious, that in such severe conditions plants are under the influence of many adverse factors (short vegetation period, low spring and autumn temperatures, droughts) and frequently they do not achieve full ripeness. Use of growth factors based on local raw material allows to solve this problem partially. Recently, humic stimulators from brown coal and peat become widely applied. They are produced by alkaline hydrolysis with, for example, NaOH or KOH. And it should be taken into account that cost of potassium reagents is considerably higher in comparison with sodium reagents and this fact is critical in their production. We have developed different modifications of humic stimulators from another accessible raw material – lake sapropel whose stocks in the Republic of Sakha (Yakutia) are huge (about 1.7 billion тones, in recalculation to 60 % humidity). For the greater efficiency, it was necessary to compare different sapropelic stimulators.

For this reason in 2001-2003 we tested two types of Na- and K-sapropelic humic stimulators on a spring wheat of *Prilenskaya 19* variety grown on small sites. Plants were treated 3 times during summer with 0.005 % solutions. Weather conditions in 2001 and especially in 2002 were very adverse for development of plants because of drought. On the contrary, 2003 was favorable (frequent rains and warm temperatures). In 2003 it resulted in the yield of wheat on the tested sites that was 2-3 times higher than the crops of 2001-2002. For this reason, we compare only, as a rule, the parameters of the treated and untreated (control) plants within a year. The grain yields of the tested sites with Na-and K-sapropelic stimulators were 14.8, 15.7 (2001); 12.1, 12.1 (2002) and 41.1, 41.4 c/ha (2003) respectively (for comparison, average grain productivity in Yakutia even in favorable years does not exceed 12.5 c/ha). In 2001 it was manifestly ($P<0.05$) above the control by 15.0 and 21.5 %; in 2002 - by 10.6 and 11.0 %; in 2003 - by 10.9 and 11.8 %. The total weight of an overground part of plants, including grains, treated with Na- and K-sapropelic stimulators made in 2001 through 2003: 52.2, 56.1; 42.9, 40.8 and 130.6, 133.8 c/ha, respectively. Authentic excess over the control was in 2001 – 10.3 and 18.6 %; in 2002 – 8.6 and 3.2 %; in 2003 – 10.9 and 13.6 %. The growth of effective germination and plant survival rate by the time of harvesting under the influence of stimulators promoted the increase of yielding ability averaging 5-10% for all years including the rise in total and productive stooling. So, in droughty 2001 excess of quantity of common and productive stalks of test variants over the control has made 11.0 and 15.4 %, and in favorable 2003 – 7.4 and 8.4 % (for Na- and K-stimulants, respectively). Besides, all treated plants, as a rule, were higher than the control variant. Parameters of a grain output and absolute weight of 1000 seeds were not so much dependent on the effect of these stimulators. The grain output from the total crop of a sheaf was within the limits of 27-32 %, but in all cases only insignificantly raised under the action of stimulants. Calculation of grains of the main ears also has not revealed an essential difference between tested and control variants.

A bit greater effect of potassium stimulators revealed in all tests, was not statistically authentic. It shows that contribution of the anionic (high-molecular) component to the general stimulating effect of preparations is much greater than of cations (Na^+ and K^+). On the whole, both sapropelic humates allow plants to use mineral and water resources more effectively per an area unit, raising their productivity that is especially important for cultivation of crops in extreme conditions of the North.

BIOLOGICAL ACTIVITY OF ENDOPHYTIC BACTERIA IN THE GENUS *METHYLOBACTERIUM*

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The endophytic role of microorganisms has frequently been determined by studying the substances that the endophytes produce. Production of phytohormones is a function found for many endophytic fungi and bacteria. Methylotrophic bacteria are frequently found to promote plant growth and some *Methylobacterium* strains have been found to produce the phytohormones cytokinins and auxins. We isolated *M. radiotolerans* from roots of potato *in vitro* plants and *M. extorquens* from bud explants of Scots pine. Inoculation of potato and pine plantlets with the two *Methylobacterium* strains increased root and shoot biomass. Furthermore, the *M. extorquens* strain increased lateral root formation of pine seedlings and the *M. radiotolerans* strain increased stem height of potato plantlets and number of sprouts when compared to controls. Also the carbohydrate and fat content of the shoots of endophyte-inoculated potato plants was higher than in the controls, and the pine endophyte affected polyamine content of the pine seedlings. When the locations of these bacteria were studied by *in situ* hybridization in the plant tissues, they were found in the cells of the pine meristem tissues and inside inner tissues and vessels of *in vitro* potato plants. It was hypothesized that the *Methylobacterium* strains are capable of producing substances beneficial for the plant tissues. Specific reaction on production of indole-acetic acid by *M. radiotolerans* was negative but the biotest with cucumber etiolated cotyledons demonstrated its capacity of producing cytokinins. Therefore, cytokinins were considered a means for the *M. radiotolerans* to directly influence plant metabolism. Detailed analysis of beneficial compounds produced by *M. extorquens* and *M. raditolerans* was performed by mass spectrometry and NMR. Among the substances detected in the media where *M. radiotolerans* had grown, cytokinins were not found. As well the Scots pine endophyte *M. extorquens* did not produce the most common cytokinins, gibberellins, or auxins. Instead, the *M. extorquens* endophyte excreted adenine and adenine ribosides in the culture medium. Adenine is sometimes used in plant meristem cultures to increase growth of plant tissue. Currently we are looking into some novel phytohormone-like substances in the culture media of these endophytes.

UKRANIAN PLANT GROWTH REGULATORS FROM IDEA TO REALITY

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During 20 years since foundation of Bioorganic Chemistry & Petrochemistry Institute of NAS of Ukraine, we have developed the whole series of high efficiency plant growth regulators of new generation.

On the basis of the fundamental research results the authors offered the hypothesis concerning universal and narrow specific action of exogenous regulators on the plant growth processes. Regulators act through changing the synthesis of phytohormones which are accounted for the recognition and regulation of gene activity. These processes promote the development and growth of plants.

Hypothesis also explains the possible mechanism of the universal action of exogenous and endogenous plant growth regulators.

New plant growth regulators activate the basic of vital functional of plants, quality of products is improved, plant stability to the diseases and damages by insects are promoted content, content of nitrates, ions of heavy metals and radionuclide in products are lowered, mutagenic action of herbicides and other anthropogenic factors is descended in two times. Developed regulators are ecologically safe. They positively influence on development of soil micro flora, growth of the root system, leaf surface and photosynthesis are strengthened, and stability to the stress factors (frost, drought, soil salinity) is promoted.

New plant growth regulators on the efficiency correspond with the best world standards, and on the technological index and the cost they are surpassed considerably. Cost of growth regulators application per 1 hectare almost to 3-7 $ USA.

The ISTC "Agrobiotech" was created in 2000 on the basis of Bioorganic Chemistry and Petrochemistry Institute for production and creation new plant growth regulators and now produce 30 items regulators for agrarian complex.

THE PRESOWING CULTIVATION BY SYNTHETIC STIMULATORS AS METHOD OF MAIZE SALT-STABILITY INCREASE IN ONTOGENESIS

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Maize is valuable agricultural crop owing to food and fodder qualities. It is possessed of malate type C_4-photosynthesis, drought-stability, high productive and so it's growing is vindicated in the South of Ukraine. At the same time it has lower salt-stability (0,6% soil salinity is critical) and thus stipulates for problem of it's growing on weak salinitive soils, which area are culminated 30% in Ukraine. In maize individual development mark out 9 men phases and 12 – women gametophytes, each of which is characterized specifical anatomical-morphological and physiological-biochemical peculiarities, calling forth differential perceptibility to different environment factors in ontogenesis.

Aim of this job is the elucidation of ontogenesis perceptibility to salinity and search of raising methods of its salt-hardiness. For solution of this object we are used: method of vegetative vessels (Studying-scientific complex for plant physiology, agrobiostation MSPU); presowing cultivation by synthetic stimulators (ivin and metiur) according to the publishing scheme, which are acted conceming regulator sort and its concentration; salinity at different individual development periods.

The receiving results are produced in table and let to assert that metiur and ivin are not resided only growing stimulative function, which is confirmed by plants morphological parameters of 2-4 variants, the first – in greater extend, but also salt-protective, than is sizeable decrease negative action of salinity in different vegetation periods.

The influence of presowing cultivation by synthetic stimulators on maize productivity depending on salinity

Experiment variant (scheme is published early)	*Plant height, sm*	*Maize ear mass, g*	*Corn mass from maize ear, g*
1	146,0±6,1	102,5±11,9	93,0±1,02
2	170,0±10,1	99,3±5,1	72,6±4,8
3	168,0±13,6	90,3±18,8	65,8±15,4
4	174,1±3,9	76,1±9,4	57,1±8,5
5	138,0±2,2	71,9±14,2	57,3±16,3
6	2*/49,5**	Ear no	0
7	3/84,0	22,9±1,48	18,8±1,0
8	5/83,6	26,5±10,9	17,1±10,2
9	1/103,0	1/29,1	1/5,7
10	2/113,5	2/17,8	2/5,2
11	138,9±16,1	28,3±8,1	18,7±7,1
12	180,1±6,1	2/63,1	2/34,5
13	158,2±6,2	1/47,1	1/36,6
14	137,1±5,1	1/38,4	1/22,3
15	130,1±10,5	2/28,1	2/17,8

*Numerator – a quantity of plants and ears, which are remained from variants (10 reiteration in each);
**denominator – mean value of proper parameter.

Thus, we are shown that growing synthetic stimulators (metiur and ivin) are most effectively at presowing cultivation in concentration 10^{-7} M, but their using is most rationally on early ontogenesis stages.

THE EFFECT OF HORMONE TO TISSUE CULTURE AND REGENERATION OF TRANSGENIC PLANT

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Salinity is a major constraint of crop productivity because it reduces yield and limits expansion of agriculture onto previously uncultivated land. Na^+/H^+ antiporters catalyze the countertransport of Na^+ and H^+ across membranes. And some evidences have proved that vacuolar Na^+/H^+ antiporters play an important role on salt-tolerance of plants. We could use the gene involved in this mechanism to modify salt tolerance of plant, which is of great significance for agricultural developments.

Based on the established regeneration system, the *AtNHX1* gene was transformed into alfalfa (*Medicago sativa*). The transformation was conducted through Agrobacterium tumefaciens-mediated callus derived from seed□cotyledon□hypocotyl and root.

In this work, we reported the effect of hormone on tissue culture and regeneration of transgenic alfalfa. Our research used MS as the basic culture mediums, changed the types of hormone combination and concentration to optimize the regeneration system and find the high frequency regeneration system.

The primary results and progress are summarized as follows:

1. Different combinative concentrations of hormones were required for different explants induced callus or somatic embryos. It was suitable contained such a combinative concentration of hormone 2,4-D 1mg/L+KT 1mg/L for hypocotyl and cotyledon, and the induction frequency was 87.2% and 83.2%, respectively. The feasible medium for callus induction from seed was the improved MS+2,4-D 2.0mg/L +6-BA 0.5mg/L, and the induction frequency was above 88.3%. The differentiation medium was MS+6-BA 1.0mg/L+KT 1mg/L+ NAA 0.01mg/L+LH200mg/L, with a differentiation ratio of 74.5%. The rooting medium was 1/2 MS with 100% rooting ratio.
2. Regenerated hypocotyls were inoculated with Agrobacterium tumefaciens strain LBA4404, which contains binary vector pBI121. Our results showed that the transformation efficiency reached up to 15.8% if using MS+2mg/l NAA+1mg/l 6-BA instead of MS. The transgenic plants were obtained by Agrobacterium tumefaciens-mediated callus induced from hypocotyls, the results of PCR and Southern analysis displayed that the exogenous *AtNHX1* gene had integrated into the genome of transgenic alfalfa.
3. We transferred the plants of transgenic type and wild type from normal MS media to that contained 0mM, 100mM, 150mM, 200mM, 250mM NaCl, respectively, and grew under artificial culture condition. After 3 weeks we found that the growth of wild-type was impaired in the MS media containing 100mM or higher NaCl concentration. The damaging effect is correlated to NaCl level in the media. In contrast to that, the transgenic plants have no obvious differences in growth subject to NaCl level from 0mM to 200mM, respectively.

***** Supported by project of Beijjng Scientific and Technological committee (2007[N]16)and Beijing Science Flat-roof Foundation. (the number of the seccion in the main topic : 6 Hormone and abiotic stress plant tolerance)

ARABINOGALACTAN PROTEINS ARE IMPLICATED IN MORPHOGENESIS IN *FAGOPYRUM TATARICUM* (L.) GAERTN. CALLUS

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Arabinogalactan proteins (AGPs) are a superfamily of structurally complex proteoglycans, implicated in various aspects of plant growth and development. AGPs are particularly abundant at the outer face of plasma membrane, in secretion and mucilages and in conditioned medium of *in vitro* cultured cells. "Classical" AGPs containing glycosylphosphatidylinositol plasma membrane anchor can interact with cell wall polymers making dynamic adhesion zones and function to link the PM to the cytoskeleton.
Previous studies have shown that morphogenic calli of tartar buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) has produced up to 30-100 – fold more extracellular AGPs as compared to non-morphogenic calli. It was proposed that such difference in AGP secretion seem to be conditioned by essential role of AGPs in keeping of morphogenic state of callus. Here, we show that the addition of $(\beta\text{-D-glucosyl})_3$ Yariv reagent (which selectively binds and perturbs AGPs) to the medium of callus-cultured cells in concentration of 250 μM do not change the growth activity of non-morphogenic callus. In contrast, in morphogenic callus, the same Yariv reagent treatment results (in dependence on 2,4-D in culture medium): 1- in 50% inhibition of proembryonal cell complex (PECCs) formation (medium with 2,4-D); 2- in completely inhibition of bud and embryoid formation but preservation, at the same time, of root formation (medium without 2,4-D). Since PECCs, as well as buds and embryoids are originated from cells of surface layers of callus (i.e. exogenously), while roots are formed endogenously, we proposed that Yariv reagent has broken the structural integrity of surface cells, thereby preventing their further differentiation or even inducing cell death.
Histologically, treated by reagent Yariv callus clumps showed a "bulging" phenotype of surficial cells. On transmission electron microscopy level, we revealed that Yariv reagent treatment resulted in hard deposition of electron dense material on cell wall, plasma membrane, and throughout intercellular spaces of surface cells. In almost all surficial and, in lesser degree, in subsurficial cells, it was shown the more or less prominent exfoliation of plasma membrane from cell wall. In certain cells, however, we revealed also a plasma membrane ruptures. Cytoplasm shrinkage and formation of apoptotic-like packets was seen mainly in bulging cells.
It is known that plant polarity and morphogenesis is controlled via integral coordination of the cytoskeleton and the cell wall functions. By the means of our results we can propose that the disruption of AGPs at the cell wall – plasma membrane interface, which tends to loss of adhesion contacts, will cause the disturbance or loss signal transduction and, finally, change cell viability, proliferation and morphogenic response of cultured cells.

The research was supported by a grant from Russian Foundation for Basic Research (grant № 05-04-49433)

BINDING OF NITROXYL BIRADICALS TO THE THYLAKOID MEMBRANES

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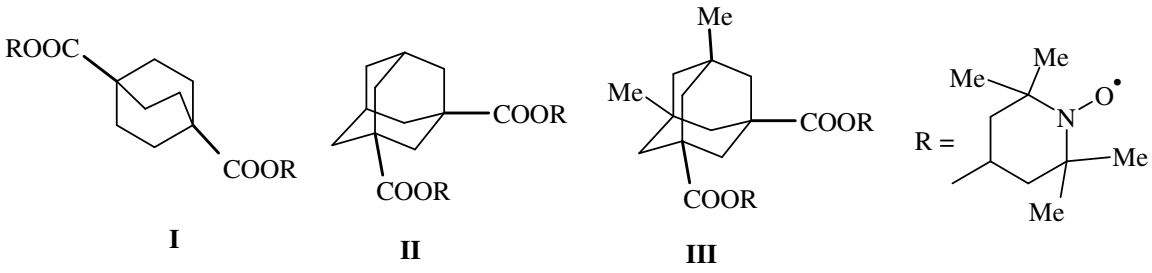
Stable nitroxyl radicals are widely used for research of photosynthetic membranes. The analysis of spectra of such radicals brings out some conclusions in relation of possible sites of localization of spin probe and roles of lipid-protein interactions. Recently, lipophilic nitroxyl radical, 1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl 1-adamantylacetate has been employed in EPR spin probe study of chloroplasts and subchloroplast fragments of different types [1]. In this study, we report the EPR properties of bis(1-oxyl-2,2,6,6-tetramethylpiperidine-4-yl) esters of 1-bicyclo[2,2,2]octane-1,4-dicarboxylic, adamantane-1,3-dicarboxylic, 5,7-dimethyladamantane-1,3-dicarboxylic acids (compounds I-III) as potential spin probes for EPR study of biomembranes. For the purpose of this work, stable nitroxyl biradicals I-III with bulky carbocyclic fragment attached to the two paramagnetic TEMPO residues have been synthesized.



I **II** **III**

The binding of spin probes I-III to membrane structures of pea chloroplasts is revealed by shape changes in EPR spectra. The high-field lines in the EPR spectrum of nitroxides in thylakoids are complex, consisting of two components corresponding to various molecular tumbling of the spin probes. Analysis of the high-field lines can be used to display the components and estimate their intensities. The broader signal, obviously, corresponds to radical bound by membrane structures. Its high-field line is located inside the resulting spectrum, because the isotropic hyperfine splitting constant is lower for the spin probe in the nonpolar than in polar microenvironment. The narrow line components of the EPR spectra can originate from the radical which is slightly bound by thylakoid membranes. The relative intensities of two components in EPR high-field lines appear different for compounds I, II, III. It was found that in the case of biradical III fraction of spin probe bound to the membranes is the much greater, than in a case of compounds I and II. Biradical III can be considered as more hydrophobic spin probe which can show stronger immobilization in biological membranes.

[1] S. M. Kochubey, A. I. Vovk, O. Yu. Bondarenko, V. V. Shevchenko, R. V. Bugas, A. K. Melnik, V. Yu. Tanchuk. *Biochemistry (Moscow)*, 2007, **72**, No. 5, 558-564.

THE REACTION OF WINTER WHEAT PHITOHORMONE SYSTEM ON MINERAL NUTRITION CHANGE

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The aim of investigation is the study of content and correlation between TAA and ABA in winter wheat root and leaves in connection with the activity of root H^+ extrusion of two varieties (Panna and Lisostep Perlina), which characterized by high harvest grain, but different by quality. The Panna variety distinguished by more high quality of seed than Lisostep Perlina.

The plants were grown to 10-15 days – old by the water culture method on the 0.5 dose of H-A mixture (control) and after sowing seed treatment by 0.4% solution of new liquid fertilizer phyziozhivlin. In 50 plants were grown in porcelain cuvezes containing 400 ml of nutrient solution, which was corrected every 3 – 4 days.

The TAA and ABA content in organs was determined using method (Savinsky, Drahovoz, Pedchenko, 1991), the redox-system activity of root cell on (Novak, Ivankina, 1986), the kinetic of H^+ extrusion was registered during 4h after transfer of the plant to the solution of 0,01 mM $CaSO_4$ + 1mM KCl (Wachmistrov, O En Do, 1993).

The results obtained have shown that the plants of studied varieties different strong by phitohormone content in organs. It has been established that the Panna variety characterized by more high TAA content in root and leaves than Lisostep Perlina variety: 423 and 615 ng/g of damp matter opposite 245 and 283 ng/g accordingly. ABA content in leaves of the Panna variety plant exceeded Lisosteep Perlina variety significant also. It makes up 278 opposite 126 ng/g damp matter. The correlation between TAA and ABA in plant organs of studied varieties was different also. Its make up 1.6 and 2.2 in root and leaves of Panna variety plant opposite 3,3 and 2,2 in organs of Lisostep Perlina variety.

Using difference genotypes of winter wheat for measurement of TAA and ABA content in root and leaves we have found the different in their reaction on act of sowing seed treatment.

It has been shown that sowing seed treatment caused decrease of TAA content in root and increase it in leaves of Panna variety plant. The ABA content in leaves increase also. Because correlation between TAA and ABA content in leaves of experimental plant almost not changed, but it decreased to 0.2 in root.

The correlation TAA/ABA in root as well as leaves of Lisostep Perlina variety under act of seed sowing treatment increased opposite to 6,9 and 10,3 accordingly.

Strong differences between studied varieties were found during study of root H^+ extrusion. It has been established that root Panna variety was characterized by more high H^+ extrusion.

So, the study of TAA and ABA content and their correlation in organs has allowed to obtain new data about difference in phytohormone system reaction of winter wheat varieties on mineral nutrition change.

It was established that Panna variety plant, which characterize by more high seed quality , distinguishe also by more high TAA and ABA content in leaves and their increase after sowing seed treatment.

ANTISTRESSFUL ACTION OF JASMONIC ACID CHEMICAL ANALOGUES ON WHEAT PLANTS

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Now the researches directed on screening of again synthesized regulators of plant growth were sharply intensified, as on modern representations, plant growing should be based on obligatory use not only fertilizers and pesticides, but also growth and stress resistance promoted substances. It is known, that structural analogues jasmonic acid are capable to stimulate growth and development of plants. The growth stimulating effect of preparations appears in nanomolar concentration (10^{-6}–10^{-9}M), that is attractive for using in stimulating of plant growth.

We analyzed influence of synthetic analogues of jasmonic acids on growth and development of wheat plants infected by root rot and septoriosis agents in laboratory conditions. The jasmonic acid applied in concentration of 10^{-6} and 10^{-9} M for soaking seeds (during 3 h). In experience variants with the septoriosis agent pieces of leaves infected spores at the rate of 10^{-6} on 1 ml. The investigated preparations differently influenced on growth parameters of wheat plant. The pretreatment by synthetic analogues of jasmonic acid results in changes degree of defeat by disease. It was revealed, that over 4 % of control wheat seedling grown in distilled water, have appeared the infected activators root rot in a strong degree. The preparation EM13 and BA66 did not affect on development of rot root whereas preparation EM90 and BA97 suppressed development of rot root. In the majority of variants of experience at increase in concentration of preparations up to 10^{-6} M the degree of seedling defeat decreased.

It was revealed, that in the control the degree of leaves defeat by septoriosis agent reached 50 %, and in variants with use of preparations in concentration 10^{-9} M the degree of defeat by septoriosis of leaves varied from 10 up to 70 %. The preparations EM31, VA66 and BA97 having morpholin groups stimulated development of disease. The application of preparation EM13, EM90, VA60 having simple amines groups resulted in discolored sites of leaves in a place of drawing pathogen, that the pathogen on them develops only superficially, not infection plants. Thus, analysis of antistressful action of preparations on wheat plants was shown, that only part of tested preparations are characterized complex protective effect against rot root and septoriosis. It is possible, that these changes are connected by structural features of preparations and according to their various degree of influence on plant defense reactions.

The work was supported by the Academy of sciences Republics Bashkortostan (Russia).

ABOUT THE MECHANISMS OF PLANT GROWTH REGULATOR ACTION AT A GENETIC

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The action of plant growth stimulators (lutidine N-oxide – ivine and its derivative) differentiationally on mRNA and rRNA synthesis as well as DNA synthesis in early postembryogenesis of embryonic axis of haricot bean seeds, which is the comfortable object of study of action of regulators on growth and development of embryos of plants, was studied (from point of detailed studied of its morpho-physiological and biochemical characteristics).

In preliminary experiments it was set, that in the earliest period of output of seeds from the state of rest (1–12 hours) the before-formated mRNA and rRNA (set aside in a supply) in late embryogenesis take part in initiation of protein synthesis. These new synthesized "early" proteins provide further "development" (adduction in action) of genetic program of progressive individual development of embryonic organism, namely, inclusion and rapid increase of syntheses mRNA and rRNA, and also biosynthesis of proteins, which takes place not due to activating of structural and ribosome genes, and due to their amplification (increase of number copies of genes). And it is possible to explain, that embryo growth at dicotyledonous plants takes place in early postembryogenesis not due to the cellular division, but due to extension of hypocotyl (or epicotyl at monocotyledonous plants), i.e. in absence of replicative synthesis DNA.

It is set, that action of plant growth regulators is unconnected with the additional increasing copies of genes, and with their physiological activation by acceleration of formation of initiator transcriptional complexes of RNA synthesis, by activating of promoters and enhancer sequences, speeds of biosynthesis of protein.

The scheme explaining plant growth regulator action at genetic level in process of plant growth and development (from embryo to formated plant) is proposed.

PLANT GROWTH REGULATORS BIOLOGICAL ACTIVITY DETERMINED BY BIOTESTING METHOD ON MOBILE MICROALGAE.

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Biological Activity was carried out on the population of microalgae, find in the steady state of development. Incubation of cells with plant growth regulators was conducted at 25ºC and standard illumination. The BA was determined at 48 hours after plant growth regulator introduction. Measuring was conducted with the using Laser Doppler Spectrometry (LDS) method. The amount of cells in unit of volume (biomass), stakes of mobile cells, mean velocity of cells movement, mean energy of cells motion and general population energy was registrated. At analysis of preparations action on microalgae examined a Biological Effect (BE) and Biological Activity (BA). Calculation of preparation BA made on expression

$K^{i}_{Eff} = \left(Par_i / Par_к - 1 \right) \times 100\%$, where Par_i is a value of vital functions parameter of microalgae population in measuring, and $Par_к$ is a value of this parameter in a control test. BA of examined preparation is determined from expression

$K^{i}_{BA} = K^{i}_{Eff} / C$, where C - is a concentration of examined matter, %.

It was shown that at the estimation of biological effect of the probed preparations in the range of concentrations from $0{,}5\times10^{-6}$ % to 2×10^{-5} % substantial changes in the coefficients of biological efficiency were not observed. Biological Effect of ***Emistim C*** with during the concentration of $0{,}5\times10^{-6}$ % $K^{Bм}_{Eff}$ = 24,7 %, and at 2×10^{-5} % K^{Bm}_{Eff} = 22,6%. However, at the analysis of ***Emistim C*** BA coefficients, the substantial diminishing in this range of concentrations - from $4{,}95\times10^{6}$ to $11{,}32\times10^{5}$, i.e. K^{Bm}_{BA} was diminished more, than in 4 times. Similar results were got and at an analysis $K^{i}_{БА}$ on a parameter "energy of population". In this case at the increase of operating matter concentration there was diminishing in two times – from $3{,}19\times10^{6}$ to $16{,}65\times10^{5}$.

Analogous researches were conducted on plant growth regulators ***Ivin.*** A range of the probed concentrations was from $2{,}5\times10^{-5}$ % to 5×10^{-4} %. K_{Eff} changed in limits from 10,4% to 33,96%. Thus also there was falling of BA with the increase of operating matter concentration. Maximal K_{Eff} of ***Ivin*** looked after during the concentration of 2×10^{-4}, for which he was equal $33{,}6\times10^{4}$. During the concentration of 5×10^{-4} % K_{BA} =$13{,}6\times10^{3}$, i.e. diminished more, than in twenty times.

Offered approach of plant growth regulators BA and BE analysis allows to analyse their effect on population of moving microalgae. So the possibility of a comparative analysis in operations of different plant growth regulators on model test-object appears also.

ENDOGENOUS PHENOL COMPOUNDS AND OIL-CONTENT OF COTTON SEEDS

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For understanding of physiological-biochemical condition of seeds the analytically directed researches are important. For representation of a complete picture of a vegetative organism, getting of information on the seeds level plays the special role. From the literary data it is known that both qualitative and quantitative characteristics defining metabolic feature of a seed are specific for each separately taken plant.

The important component is presence of activators and inhibitors adjusting a metabolism, defining specific specificity and efficiency of plants. In this connection, a cotton-plant with presence of endogenous inhibitor of reaction – gossypol (2,2-di, 1,6,7-trioxy-3-methyl-5-isopropyl-8-aldehyderonaphthyl) presents special interest. Gossypol – specific for Gossypium type compound of polyphenol nature. It is being formed from the first days of cotton growth and is present in all vegetative and generative bodies. As the molecule has numerous functional groups (hydroxyl, aldehyde), depending on environment where the reaction takes place, it can be an acceptor and the donor of electrons. Gossypol has rather high oxidation-reduction potential (B_o=0,77) close to oxygen potential (B_o=0,82) and participates in cell respiration processes as one of key interim electron carrier.

Due to this, it was important to conduct parallel research between the presence in the cotton seeds extract of phenol compound and free fat acids (in particular, oleic acid).

The cotton-plant Gossypium hirsutum (L.) of Mehrgon and Gissar sorts were researched. It was revealed that oil allocated from seeds of two sorts is characterized by presence of phenol compounds which have high toxicity. Research of physical-chemical constants (saponification number, iodine number) of the studied sorts revealed that according to these indicators Mehrgon and Gissar sorts differ from each other both by having free fat acids and phenol compounds.

The following regularity was revealed: the more maintenance of free fat acids is, the more is maintenance of phenol compounds, that negatively influences on quality of cotton oil. It is known that lipogenesis in plants and chemical composition are very conservative signs that are being controlled at the genome level. In this connection, receiving of cotton sorts with reduced amount of gossypol and other phenol compounds influencing the metabolic processes connected with formation of fat acids responsible for oil content is important.

Due to this, during development of effective ways of an estimation of biochemical indicators of cotton oil-seeds quality, it is necessary to consider proportion of phenol compounds and fat acids presence in them.

HORMONAL REGULATION OF PLANT RESPONSE TO WOUNDING

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Plant that are repeatedly wounded are stunted and show enhanced resistance to pathogens and pests. This is associated with the accumulation of the hormone jasmonate. Notably, exogenous application of of jasmonate stunts plant growth, and enhances resistance to pathogene and pests. As expected, application of jasmonates to plant re-programmes transcription. A challenge has been to understand the link between jasmonate application and altered gene expression. Recent progress has indentified mutants that define the key players in the pathway that links perception of wounding to expression of the response. The key players in the jasmonate perception-signal pathway are: JAR1; COI1; MYC2; and JIN3. A new class of proteins – the JAZ proteins – link the jasmonate perception pathway to the execution of jasmonate responses.

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2nd International Symposium
"Plant Growth Substances: Intracellular Hormonal Signaling and Applying In Agriculture"

Abstracts

Recommended to publication by academic council of
Institute of Bioorganic Chemistry and Petroleum Chemistry NAS of Ukraine
Protocol №7 03.09.07

Mechanical was prepared by Iakovets O.V., Kurchii B.A., Kravets V.S.
Cover design: Davnya E.O., Iakovenko O.M., Pokotylo I.V.

In cover: St. Sophia Cathedral
Photo: Iakovenko O.M.

Підп. до друку 10.09.07. Формат 60×84[1]/8. Папір офс. Гарнітура Times.
Спосіб друку – ризографія. Ум. друк. арк. 16,24. Зам. № 7-214. Наклад 150 пр.

НТУУ "КПІ" ВПІ ВПК "Політехніка"
Свідоцтво ДК № 1665 від 28.01.2004 р.
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