

Session 2:

Role of hormones, protein kinases/phosphatases and cytoskeleton in signal transduction

EFFECT OF BRASSINOSTEROIDS ON TUBERIZATION AND STEM GROWTH OF POTATO PLANTS IN VITRO

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Brassinosteroids (BS) are endogenous growth promoting plant sterol hormones. Effects of BS on growth and morphogenesis are well documented in many plant species including potato plants (Khripach et al., 2000). We have found earlier (Aksenova et al., 2005, 2009) the close negative correlation of biomass partitioning between stems and tubers in potato plants cultured in vitro. The aim of the present work was to compare the effect of BS on stem growth and on tuberization in potato plants of two genotypes differing in growth parameters. Experiments were performed with *Solanum tuberosum* cv. Désirée (non-transformed plants, NP) and the phytochrome B enriched line transformed with the *Arabidopsis PHYB* gene under control of the 35S CaMV promoter (phytochrome transformants, PT). In vitro cultured PT plants have more pronounced shoot growth but weaker tuberization than NP plants. Single-node NP and PT stem cuttings were planted in the agar-solidified MS medium containing 5% sucrose. The culture medium was whether lacking hormones (control medium, CM) or supplemented with 24-epibrassinolide (EB) or 28-homobrassinolide (HB). The comparison of various EB- and HB-doses (0.005 – 0.1 mg l⁻¹) showed that the most effective concentration for both BS was 0.01 mg l⁻¹ (~ 2 · 10⁻⁸ M). At this concentration BS markedly stimulated the early initiation of tubers at the expense of stem growth. Both BS forms acted similarly though EB seemed to be more effective than HB. PT-plants were more sensitive to BS-treatment than NP-plants. After 2 weeks of cultivation, stems of NP-plants were 2.2-times shorter while stems of PT-line were 4.3-times shorter than those in CM. At this time the tuber initiation in plants grown on EB-medium compared to plants grown on control medium rose by 130 and 170% in NP- and PT-plants, respectively. At the end of the experiment differences in tuber frequency decreased as a result of gradual increase (approaching to 100%) in tuber initiation in all plants under study. The data obtained show that BS signaling might take part in the regulation of sink-source relations between stems and tubers in potato plants.

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PHYTOHORMONES'S SYNTHESIS ABILITY OF PATHOGENIC FOR LEGUMES BACTERIA OF *PSEUDOMONAS* GENUS *IN VITRO*

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Phytohormones play the important role as regulators of plant growth and development. There are three groups of plant growth substances: stimulator (auxins, cytokinins and gibberellins), inhibitors (ethylene and abscisic acid) and some hormone-like compounds. Therefore, microorganisms associated with plants are able to synthesize phytohormones themselves. Some classes of phytohormones, produced by phytopathogenic bacteria, determine the development of the initial stages of pathogenesis, leading to plant's infection. The subject of research was analysis of some phytohormones production by pathogenic for legumes bacteria of *Pseudomonas* genus *in vitro*.

The objects of research were typical representatives of pathovars, which could cause various types of necrosis lesions of legumes. We also used classical polyphages – *P. syringae* pv. *syringae* B-1027, which could induce disease more than 50 plant's species, including legumes. The typical tumor inducing strains *P. savastanoi* pv. *savastanoi* 9174 was used as a good producer of auxins and cytokinins. It has been determined that all strains of pathogenic bacteria produced a wide spectrum of auxins: the indole-3-acetic acid and indole-3-carboxylic acid complex, indole-3-carboxylaldehyde, hydrazine indole-3-acetic acid. The highest level of total auxins production was founded not only in tumor inducing but also in classic polyphages strain. All strains of phytopathogenic bacteria produce low contents of cytokinins, such as: zeatine-riboside, isopentenyl-adenine, isopentenyl-adenoside. The high level of total cytokinins production was detected only in tumor induced stain. We also established the ability to synthesize abscisic acid by all phytopathogenic bacteria strains, that binds with high level of its virulence. It should be also noted the ability of some strains to produced the significant amounts of ethylene. According to research phytohormones, produced by phytopathogenic bacteria, significantly impact on their strategy of pathogenicity.

BRASSINOSTEROIDS IN REGULATION OF OXIDATIVE STRESS DEVELOPMENT UNDER ABIOTIC STRESS ACTION

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The past decade has seen a tremendous increase in BR research, which has demonstrated that BRs capable of improving plants resistance to a range of biotic and abiotic stresses. By investigating the mechanisms of BR action we showed that they are tightly connected with the reactive oxygen species metabolism and can regulate cellular level of ROS, thus promoting defense responses. It was shown that under osmotic stress conditions BRs activated catalase and superoxide dismutase enzymatic antioxidants. Interestingly, guaiacol peroxidase was inhibited in similar conditions in *A.thaliana* plants. In addition, BR compounds also increased cellular levels of non-enzymatic osmoprotectants and scavengers of reactive oxygen species, namely proline and glutathione. We analyzed concentrations of hydrogen peroxide in wild-type *coll* plants and BR-signaling mutant *bak1-1* and demonstrated that *coll* plants maintained higher level of hydrogen peroxide than *bak1-1* plants that points at BRs involvement to both stimulation of ROS production and activation of antioxidant systems. Transgenic *bak1-1* plants were also characterized with reduced resistance to abiotic stresses.

It was also shown that under stress conditions BRs facilitate regulation of alternative oxidase in plant mitochondria *in vivo* thus preventing ROS generation and oxidative stress development. Although, BRs had no effect on alternative oxidase activity in BR-signaling mutant *bril-5* it indicates implication of receptor-mediated pathway into regulation of alternative oxidase. Treatment with brassinazole – an inhibitor of BRs biosynthesis – significantly reduced activity of alternative oxidase while BRs supplementation led to increase of alternative respiration.

In consent, BR compounds were shown to impair ROS metabolism by regulation of antioxidant systems and alternative oxidase thus maintaining plant cell homeostasis.

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THE ROLE OF 24-EPIBRASSINOLIDE IN PEA LEAF SENESCENCE

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During senescence, leaf cells experience dramatic changes in metabolism. The most striking phenotypic change is the yellowing of the leaf caused by the preferential breakdown of chlorophyll and chloroplasts. The loss of the photosynthetic pigment chlorophyll and the breakdown of the structural integrity of the chloroplasts attenuate energy-requiring anabolic events such as photosynthesis and protein synthesis. De novo synthesis of specific proteins is also required for senescence. Although senescence occurs in an age-dependent manner in many species, the initiation and progression of senescence can be modulated by a variety of environmental factors. It is known that internal factors such as plant growth regulators, reproduction, and cellular differentiation also influence senescence (Woo et al., 2001). Among these internal factors, plant hormones have been characterized most thoroughly at the molecular and physiological levels. In this study, senescence symptoms induced by plant steroid hormone 24-epibrassinolide (EBR) were examined in pea leaves at the molecular level. We show that exogenous EBR (10^{-12} M) in detached leaves, incubated on light for 15 days, reduced speed of the Chl loss. By the methods of two dimensional electrophoresis and subsequent by immunoblotting with application of monoclonal anti-phosphotyrosine antibodies we revealed the epibrassinolide-induced effect on the level of tyrosine protein phosphorylation of senescent leaves. Also by method of electron microscopy we showed that EBR changed RUBISCO localization in senescent chloroplasts. The data obtained allowed us to suppose the protective role of EBR in senescent photosynthetic tissues.

USING OF TRIFLUOPERAZINE, AN INHIBITOR OF CALMODULIN-DEPENDENT PROTEIN KINASES FOR IMPROVEMENT OF AGROBACTERIUM-MEDIATED PLANT TRANSFORMATION

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Protein kinases and protein phosphatases are key compounds of plant defence transduction against different pathogens. In particular after the plant recognizes the pathogen, it responds by activating of mitogen-activated protein kinases (MAPK) that form the basic system of plant defence (Zaltsman et al., 2010). Therefore, the actual direction of research increasing the efficiency of *Agrobacterium* transformation is the use of compounds that inhibit the protective reaction of plants. It was shown recently that the application of inhibitors of serine-threonine protein kinases and protein phosphatases during *Agrobacterium*-mediated transformation increases significantly the efficiency of white pine embryos transformation. For example, 150 μM trifluoperazine, an inhibitor of Ca^{2+} -calmodulin-dependent protein kinases, promoted 2.5-fold increase of transformation frequency after embryos treatment as compared to control (Tang et al., 2007). It was suggested that trifluoperazine inhibits Ca^{2+} -calmodulin-dependent stimulation of 3',5'-cyclic nucleotide phosphodiesterase that plays an important role in the protective reactions of plants during agrobacterial infection.

To improve *Agrobacterium*-mediated transformation of leaf disk explants of *Nicotiana tabacum* broad range of trifluoperazine concentrations (10-300 μM) and treatment duration (10 min - 48 h) were tested. *Agrobacterium tumefaciens* LBA4404 strain bearing the pBIN20 vector construction with a selective marker gene *nptII* conferring kanamycin resistance was used in experiments. Trifluoperazine was added directly to nutrient medium for *Agrobacterium* and tobacco explants co-cultivation. As explants an aseptic young leaf disks, which size varies from 1.5 to 2.5 cm^2 were used. All experiments were performed in three replicates. It was found that explant treatment with trifluoperazine in high micromolar concentrations (200 and 300 μM) caused the tobacco leaf explant necrosis in 7 days after transformation. But leaf disk co-cultivation with agrobacteria in the presence of trifluoperazine in lower concentrations (50, 100 and 150 μM) resulted in 50, 40 and 20% transformation frequency respectively. However, the most effective concentrations of trifluoperazine, 10 and 25 μM , increased the transformation efficiency to 98%, while in the control samples it has not exceeded 90%. The transgenic nature of regenerated plants are investigated by PCR analysis.

BRASSINOSTEROIDS IN THE SYSTEM OF LEGUME METABOLISM INTEGRATION

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It is generally accepted that brassinosteroids fulfill the integrative functions in plants and like steroidal hormones of animals and humans are involved in ensuring of plant organism integrity. This paradigm is quite defensible due to the constant interest to steroidal phytohormones and, as a consequence, to new data that brassinosteroids are one of the most common regulation systems of plant metabolism. In such a context, it is important to study the role of brassinosteroids in integration of physiological processes of plant host and nitrogen-fixing bacteria of *Rhizobium* sp. in the formation of legume-rhizobium symbiosis as well as in the regulation of fruit formation and nonspecific resistance of legumes.

In this work we investigated the influence of epibrassinolide (EBI) on Lupine and Soybean plants in phases of flowering and complete ripeness. The plants were treated by the presowing soak in EBI solution (10^{-9} M) and grown under conditions of field experiments or in Mitcherlich containers. In the latter case the soil contained Cd (20 mg/kg) or 0.1 M NaCl. The soil contained also the clean culture of *Rhizobium lupini* or *Bradyrhizobium japonicum*. It has been shown that EBI promoted the nodulation and activity of root/nodule lectins; metlegoglobinreductase; increased legoglobin level with altered spectral characteristics; nitrogen-fixing activity of productive *Rhizobium* cultures up to 80% compared with the symbiotic activity of spontaneous rhizobial microflora. Moreover, the reduction of pollutant toxicity and the phytopathogenous fungi *Colletotrichum* sp. injury under natural infectious background took place in EBI-pretreated plants. It can be explained by temporary activation of trypsin inhibitors and root endogenous lectins. In all cases we observed EBI-induced increasing of the mass of 1000 seeds on average up to 10-14%. It has been expected that brassinosteroids accomplish general regulation of functional activity of micro- and macro-symbiont at the forming of an effective symbiotic nitrogen-fixing system, providing positive action on the components of nitrogenase complex, proteolysis system, metabolism of proline and endogenous lectins.

BIOINFORMATIC ANALYSIS OF THE MOSS KINOME: GOING DOWN THE STAIRWAY OF EVOLUTION

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Protein phosphorylation in eukaryotic cells is considered to be a central mechanism for regulation and cellular signaling. In animals, proteins phosphorylation realized by serine/threonine (S_TKc) and tyrosine (TyrKc) protein kinases, which have a common evolutionary origin [PMID:19369195]. In flowering plants, wide range of S_TKc kinases as well as kinases with dual (STYKc) specificity was identified, but in opposite to animals, existence of TyrKc kinases are still ambiguous. Nevertheless, the relative abundances of pS, pT, and pY in *Arabidopsis thaliana* were estimated to be 85.0, 10.7, and 4.3%, which are strikingly close to the human phosphoproteome profile [PMID: 16429265, 19162527, 21628997]. Also, it was demonstrated the importance of tyrosine phosphorylation for cytoskeleton regulation in plants [PMID: 20167106, 18800224]. At the same time, our bioinformatic search demonstrate that among 1024 protein kinases of *A. thaliana* only 3 match HMM profiles of canonical tyrosine kinases. Thus, tyrosine phosphorylation in *A. thaliana* is realized by dual specificity protein kinases mainly. The purpose of our study of *Physcomitrella patens* "draft" kinome was to clarify some aspects of protein kinases evolution and, in particular, loss of typical tyrosine kinases, going down the stairway of evolution of higher plants.

Physcomitrella patens kinome was reconstructed based on information from UniProtKB, GeneBank, JGI and COSMOSS. We analyzed 2743 amino acid sequences and identified 462 protein kinases which have unique genes and amino acid sequences. Multiple alignments and NJ-trees were reconstructed using ClustalX and MEGA5. Based on comparison with HMM-profiles of kinase domains, it was proved that among the 462 protein kinases, 407 belong to S_TKc kinases, 45 have dual specificity, 9 (UniProt: A9RMB5, A9S2K7, A9T6C5, A9TJD7, A9RVT3, A9S6F8, A9TVK7, A9SWW2 and A9S9S5) belong to the potential TyrKc kinases and 1 (UniProt: A9TMS2) protein kinase has an unknown substrate specificity and function. We identified homologs of S_TKc kinases (STK, AGC, CAMK, TKL, STE, CMGC, CK1) and dual protein kinases TTK, phosphorylating microtubules and regulating cell division in animals. The reconstruction of 3D-models and molecular dynamics simulation of potential tyrosine kinases and 21 serine-threonine protein kinases (similar to animal Aurora, CKI, CKII and CDK) were performed. Constructed models demonstrate correct stereochemistry and structural similarity with appropriate animal and plant homologs.

In summary, results obtained for *P. patens* and *A. thaliana*, confirm the theory that loss of "mammalian" type of tyrosine kinases in plants due to alternative signaling pathways [PMID:21315387] occur the early plant evolution. This is the most probable reason that now Tyr phosphorylation in higher plants executes mainly by dual-specificity protein kinases.

MANIPULATION OF ENDOGENOUS HORMONE STATUS AND TUBER FORMATION IN POTATO PLANTS

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Potato is one of the most important food cultures, therefore extensive studies of this plant have the goal to increase tuber yield and plant resistance against unfavorable environment. Previous works of our laboratory (Romanov et al., 2000; Aksenova et al., 2000) showed that the application of different phytohormones influenced tuber formation in potato plants. To better understand the role of auxins, cytokinins and gibberellins in the regulation of potato ontogenesis we have produced new forms of transgenic potato with modified hormonal status. These transgenic forms were checked for the changes in their development, especially as regards tuber formation.

We used the agrobacterial method for transformation of 8-week-old potato plants cv. Désirée with target genes under control of the B33 patatin promoter which provides adjacent gene expression in tuber. For creating plants with elevated auxin level we used auxin biosynthesis gene *tms1* in plasmid construct *B33::tms1*. Plant transformation was carried out by a Prat' procedure. Potato leaves were cocultivated with *Agrobacterium* in liquid Murasige-Skoog (MS) medium during 48 hours in the dark, then they were transferred onto solid media containing phytohormones: 5 mg/l α -NAA (auxin), 0.1mg/l 6-BAP (cytokinin), and antibiotics: kanamycin (50 mg/l) and cefotaxime (250 mg/l). Explants were cultivated at 26°C under long day (LD) conditions. Every 2 weeks explants were transferred to fresh media. First calluses arose after 2.5 weeks, and after 4 weeks first shoots appeared. 10 weeks after transformation many of shoots produced roots. These lines were tested by PCR method and 2 lines phenotypically different from control plants were selected among 20 clones containing the target construction. Free indole-3-acetic acid (IAA) was measured in tubers by HPLC-MS/MS method and 1.5-fold increase in the IAA level in transformed tubers over control ones was observed.

In experiments in vitro (5% sucrose) in the dark both selected lines formed tubers a week earlier than control plants. Under LD conditions 25% of transgenic plants formed axillary tubers which were further transformed into shoots. Potato transformants grown on media with different (1, 3, 5 or 8%) sucrose were able to form tubers earlier than controls, and at a lower sucrose content the number of tubers formed after 8-week-cultivation 2-3-times exceeded that of control. Under short day conditions or in the dark plants of all genotypes formed similar quantity of tubers after 8-week-cultivation but the weight of transformed tubers per plant was 1.5-3-fold higher.

Potato plants were transformed by the same method with gene constructs increasing (pK7WG-B33:gIPT3) or decreasing (pK7WG-B33:gCKX1) level of cytokinins, and also increasing (pK7WG-B33:gGA3ox3 and pK7WG-B33:gGA20ox1) or decreasing (pK7WG-B33:gGA2ox1) level of gibberellins in tubers. Obtained lines were tested with PCR method and clones containing target constructions were selected for further investigations.

Results of our work clearly demonstrate that the process of tuber formation in potato plants can be manipulated by means of organ-specific change of hormonal status.

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PHOSPHONATE INHIBITORS OF *YERSINIA* PROTEIN TYROSINE PHOSPHATASE

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The protein tyrosine phosphatase termed YopH is a key outer membrane protein H secreted by pathogenic bacteria *Yersinia*. The species of these bacteria may cause several human diseases ranging from gastrointestinal syndromes to bubonic plague. In infected cells, YopH can dephosphorylate multiple proteins such as focal adhesion kinase and focal adhesion protein p130Cas to disrupt signaling pathways and to escape immune responses. Natural reservoirs of *Yersinia pestis* still exist and there is a risk of outbreak of the diseases. Because the YopH, one of the most active PTPs known, is an essential virulence factor of the *Yersinia pestis*, there is growing interest in developing inhibitors of this enzyme.

This research was undertaken in order to evaluate the properties of several organic phosphonates towards recombinant protein tyrosine phosphatase from *Yersinia enterocolitica*. Among series of the compounds represented as heterocyclic,¹ aliphatic and aromatic derivatives, the phosphonate and methylenebisphosphonate substituted calix[4]arenes showed the highest affinity for the enzyme with inhibition constant in the low micromolar range.²

For elucidation of the molecular mechanism of the inhibition the tested macrocyclic compounds were docked computationally to the active site of the *Yersinia* protein tyrosine phosphatase. The docking calculations for all of macrocyclic inhibitors reveal that p*K*_i values obtained from Δ*G*_{doc} are in good correlation with the experimentally determined activity data. The studies showed similar binding mode for calixarene inhibitors covering the active site, with location of the phosphonate residues around the entry of the phosphotyrosine binding cavity of the enzyme. This mechanism provides molecular basis for understanding the enzyme–inhibitor interaction and may be useful for the development of novel inhibitors of the *Yersinia* protein tyrosine phosphatase.

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THE PARTICIPATION OF THE Ca^{2+} SIGNAL SYSTEM IN THE REGULATION OF IAA-INDUCED ADVENTITIOUS ROOTS FORMATION.

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The auxin (IAA) promotes the process adventitious root formation through the regulation of cell dedifferentiation to reestablish the new apical meristem. Although a variety of components of auxin signal transduction have been identified, the molecular mechanism and intermediates underlying the signal transduction of auxin-promoted root formation remains a major goal for a large number of researches. In plant cells no second messenger has been demonstrated to respond to more stimuli than calcium. It is well known that auxins induce changes in cytosolic Ca^{2+} ; however relationship between these two effectors is unclear and inconsistent. Besides, it is not so much known about a role of Ca^{2+} in an adventitious organogenesis. Thus, though the Ca^{2+} signal system is one of the most studied the question of its participation in the root formation process, still remains studied insufficiently.

In our work the involvement of Ca^{2+} in IAA-induced adventitious roots formation on thin-layer explants from buckwheat hypocotyls was investigated. In the course of a rooting the stages on sensitivity to auxin were allocated, namely: from 0 to 1 hour of culture, when presence of IAA at the medium wasn't necessary and from 1 to 20 hours culture, when presence of the hormone was necessary. Such periods weren't revealed for calcium because lack of an ion in the medium even within 15 minutes led to reduction of quantity of roots and an ion should be present before hormone addition.

Adventitious root formation was reduced by L-type Ca^{2+} -channel blockers (verapamil, diltiazem) and compounds affecting the release of intracellular Ca^{2+} from the vacuole (ruthenium red, neomycin). Thus, IAA-induced root formation depends on the availability of both external and internal Ca^{2+} pools. The application of calmodulin inhibitor (chlorpromazine) decreased the number adventitious roots. By contrast, other calmodulin antagonist (fluphenazine) increased the root number on the explants. The fluphenazine effect in this case is directed on increase of the endogenous cGMP level through the inhibition of the phosphodiesterase (PDE) activity because the complex Ca^{2+} -calmodulin was identified as plant PDE effector. Collectively, our results are present strong evidence to unravel part of the signaling cascade which involved cytosolic Ca^{2+} and operates downstream of IAA to trigger the adventitious roots formation.

PHYTOHORMONES IN FUNGI *AGARICUS BISPORUS* AND *PLEUROTUS OSTREATUS* AND THEIR COMPOSTS

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As a result of mankind unreasonable activities in the period of an increasing pressure on the environment the problem of looking for some sources of effective, ecologically pure preparations characterized by a high growth regulating activity meant to improve the agricultural plant growth and development characteristics, to raise their productivity, resistance to diseases, to increase the period of their storage etc. is very urgent. One of the promising scopes of searching is the field of edible fungi cultivation when the compost after their growth period may be used in the crop production as organically hormonal complexes. Our studies were aimed at the determination of the fungi and composts phytohormonal status following their cultivation that will make it possible to find the most promising sources of biologically active substances during the development of complex preparations for the plant production. The objects of studies were higher basidial fungi *Agaricus bisporus* and *Pleurotus ostreatus* as well as composts of wheat straw and sunflower wastes after their cultivation. Quality and quantity analyses of phytohormones were done using the highly effective liquid chromatography according to (Methodical Recommendations on phytohormones measurement, 1988)

It was shown that in basidium of *Agaricus bisporus* the content of free IAA was higher while in *Pleurotus ostreatus* there prevailed the bounded form and in composts it was the free form that quantitatively prevailed besides, in wheat straw composts these levels were by 30% higher than in those of sunflower wastes. As for ABA in fungal fruit bodies and composts free forms dominated quantitatively. High levels of cytokinins were observed both in basidium and composts following the cultivation. The cytokinin content in fruit bodies of *Agaricus bisporus* was almost ten times higher than that of *Pleurotus ostreatus*. Fruit bodies of *Agaricus bisporus* and *Pleurotus ostreatus* were found to contain more IAA, ABA and cytokinins than composts after their cultivation. Thus, not only fungi but their composts as well have high contents of biologically active substances – stimulating phytohormones and ABA.

THE POSSIBILITY OF INVOLVEMENT OF SEVERAL PROTEIN KINASES IN FLOWERING INDUCTION OF SHORT-DAY PLANTS

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Induction of flowering is a key event in ontogenesis of photoperiodic sensitive plants. Wide spectrum of components of this induction does not exclude significant role in this transduction passway of photoperiodic signal through protein kinase (PK) systems. The aim of this work was to study protein kinase C (α and η) isoforms, MAP kinases (ERK1/2, JNK/SAPK, p38) in leaves and stem of different short-day plants (Nicotiana tabacum L. M. Mamoth, Perilla crispa L. and Chenopodium rubrum L.) during photoperiodic induction. Protein kinase expression was studied by immunoblotting method using monoclonal antibodies for different PK (Sigma, USA). Secondary antibodies and reagents for chemiluminescent analysis were obtained as a kit of ECL (Amersham Life Science, USA). As a result of the conducted work, flowering induction of short-day tobacco, Perilla and Chenopodium plants led to activation of Ca^{+2} dependent α -PKC expression. Also, we have found increase of MAP kinase p38 expression during flowering induction in Chenopodium rubrum L. The study of MAP kinase expression in plants flowering induction will be continued.

PHYTOHORMONAL COMPLEX OF WILD ORCHIDS AND THEIR INTRODUCTION INTO THE CULTURE *IN VITRO*

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The such bio-ecological characteristics as a low competitiveness and mycosym-biotropism as well as specific pollination, inbreeding processes of small in number populations, decorative and attractive features make species family *Orchidaceae* the most impressive group of plants. Today the problem of wild-growing orchids protection in the temperate zone is very important and that is why the preservation of rare orchid species gene pool requires their more global and detail studies. Along with the traditional techniques of plant preservation *ex situ* biotechnological methods used for these purposes are becoming more and more important.

These work deals with studies on the components of the wild orchid phytohormone complex at the various stages of ontogenesis and with the elaboration of approaches to their introduction into the culture *in vitro*. As a result there were obtained callus cultures of vegetative and generative organs of 11 wild orchid species. It is the first time when there has been found the interrelation of callusogenesis intensity of orchid vegetative and generative organs explants and the content and ratio of the phytohormonal complex components at the specified stages of ontogenesis that must be taken into account in the elaboration of methods of this species microclonal reproduction.

It has been shown that during ontogenesis the cytokinin, IAA and ABA content between the orchid organs changes and the ratio of active and bound phytohormone forms varies. During the transition to the reproductive development the content of IAA and cytokinins in the orchid generative organs increases and that of the vegetative organs decreases. At the specified stages of ontogenesis there has been observed the interrelation between the content of orchid intact organ endogenous phytohormones and callusogenesis intensity of these organs explants. The maximum frequency of callusogenesis under these conditions of cultivation has been shown to be typical of the orchid generative organs since they are characterized by increased content of endogenous cytokinins and IAA and low level of ABA. It has been shown that callus plants might further on be practically used for renewal and preservation of rare and endangered orchid species of the Ukraine flora.

THE ROLE OF NITRIC OXIDE IN AUXIN SIGNAL TRANSDUCTION

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Auxin is one of the first discovered “canonic” phytohormones. Its signal transduction has been studied for a long time, and data have been accumulated indicating the participation in this process of a number of second messengers, including nitric oxide and superoxide. In 2005, the auxin receptors F-box proteins were discovered: TIR1 and other AFB-proteins. Therefore, it became necessary to reconsider the role of second messengers in the transduction of auxin signal.

To study the role of nitric oxide (NO) in auxin signal transduction we used 3-day-old seedlings of *Arabidopsis thaliana* (L.) Heynh. wild type, as well as 3-day-old seedlings of pDR5::GUS transgenic *Arabidopsis*. By means of fluorescence microscopy a 3-fold increase in NO content in root cells treated with auxin (5 μ M) for 15-30 min was documented. The NO identification was performed by the cell-permeant NO-sensitive fluorescent probe 4,5-diaminofluorescein diacetate (DAF-2 DA). Determination of the fluorescence intensity was measured in the zones of root cell division and elongation. Cell-permeant NO scavenger cPTIO, hydroxocobalamin (Cob) and specific nitrate reductase inhibitor sodium tungstate inhibited the effect of auxin by 52, 56 and 50%, respectively. The nitric oxide donors SNP, NOR-3 and S-nitrosoglutathione promoted the expression auxin primary response genes exemplified by *IAA3* (increase by 120-140%), *IAA5* (increase by 320-350%) and *IAA9* (increase by 100 to 180%). cPTIO, Cob and sodium tungstate inhibited the auxin effect on the expression and *IAA3*, *IAA5* by 85-95%, and have no effect on the expression of *IAA9*. Kinetic-inhibition analysis on a pDR5::GUS seedlings showed that NO is involved in the early stage preceding the transcription. The actinomycin D was used as RNA polymerase inhibitor. The 50% inhibition of the auxin effect comes after administration of cPTIO and Cob in 7.5 and 10 min respectively, while after administration of actinomycin D only in 15 min. Pharmacological analysis with the use of pDR5::GUS seedlings confirmed the results of the gene expression study. SNP and NOR-3 increased the GUS-activity by 20 and 36%, respectively. cPTIO, Cob and sodium tungstate inhibited the effect of auxin by 74, 76 and 55%, respectively. The nitric oxide donor SNP did not overcome the negative effect of proteasome inhibitor MG-132 on auxin signal transduction. These results suggest the participation of nitric oxide in the 26S proteasome-dependent auxin signaling.

PHYTOHORMONES IN THE DEVELOPMENT OF *EQUISETUM ARVENSE* L

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Study on the regulatory systems and on the hormonal one ensuring plant organism growth, development and reproduction in the first place is one of the most important problems in the modern phytobiology. These genetically programmed processes and their regulation have been investigated using mostly higher plants. Information concerning the phytohormones role in growth and development of spore plants is scarce and limited only by the data about their identification or influence on the development in culture *in vitro*. The aim of this work was to study the patterns of changes in content, ratio and composition of IAA and ABA during the development of sporophyte of summer field horsetail – *Equisetum arvense* L..

The objects of studies were vegetative sprouts and organs (internodes, lateral branches, ring leaves of shoot, rhizomes) of horsetail sporophytes collected during various periods of their development in Kiev and Zhytomir regions. A quality and quantity analyses of phytohormones were carried out using HPLC (Methodical Recommendations, 1988).

There was found a direct relation between the growth intensity of overground (upper and lower internodes, rings of lateral branches of the first order with leaves) and inverse relation of underground (rhizome) parts of the vegetative shoot of field horsetail with levels of IAA and ABA. The formation and development of lateral branches of the second order in nodes of branches of the first order were shown to be associated with a higher content of IAA forms (free and bounded) and ABA (bounded) both in the overground and underground parts of a vegetative horsetail shoot and that considerably exceeded their content in ontogenetically younger plants. In mature vegetative plants a decrease in shoot growth intensity was accompanied by an increase in the content of studied phytohormones in rhizomes mostly due to conjugated forms. Thus the data obtained indicate that phytohormones (IAA and ABA) play a regulatory role in growth and development of vascular spore and higher plants.

DYNAMICS OF PHYTOHORMONES IN THE BLACK SEA BROWN SEAWEED *CYSTOSEIRA BARBATA* DURING THE VEGETATION PERIOD

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Replacement of chemical fertilizers, synthetic growth regulating substances and pesticides by natural analogues in the national economy is an important problem of modern researches. A great variety of algae, first of all macrophytes, arouses interest for their investigation as promising producers of physiologically active substances during the development of ecologically pure plant growth regulators. The most productive dominant species of the Black Sea phytocenosis is a brown seaweed *Cystoseira barbata* (Good et Wood), whose greatest biomass accumulation was observed during the autumn and spring periods of vegetation in the phase of the most intensive development of its reproduction organs. For the purpose of the most effective usage of natural populations of *Cystoseira* and its wastes in order to make preparations with a high physiological activity there should be determined optimal periods of its development in terms a maximum phytohormones accumulation. We studied the seasonal dynamics of the phytohormones content in an intact thallus of the Black Sea weed *C. barbata* from the natural populations found and collected in bays of Sevastopol. Phytohormones were measured using the well-proved techniques of highly effective liquid chromatography involving IAA and ABA preliminary purification by means of acid-alkaline trans-extraction and thin layer chromatography, cytokinins – by means of ion exchange chromatography in the DOWEX 50Wx8 columns in H⁺-form 0,1N by ammonia and by means of thin layer chromatography in the combination of solvents, gibberellins – by means of bioassays (Methodical Recommendations, 1988).

It was shown that for the whole period of seaweed vegetation its extracts displayed some insignificant activity of gibberellin-like substances. In autumn (prior to slowdown of weed growth) and in winter the level of IAA decreased. The highest levels of cytokinins in *Cystoseira* occurred in winter while in spring and in autumn (during its intensive growth) their content decreased half as much but it was higher than that in summer samples. In *Cystoseira* winter samples, for example, the cytokinin content was about 300 ng/g of fresh substance mass that considerably exceeded their quantity found in higher plants. The obtained results indicate that the winter thallus of *Cystoseira* contained the greatest quantity of phytohormones mostly of cytokinin nature.