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.

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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.