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].

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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.

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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.

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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.

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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.