

SESSION 7:

Hormones and synthetic plant growth regulators in agriculture

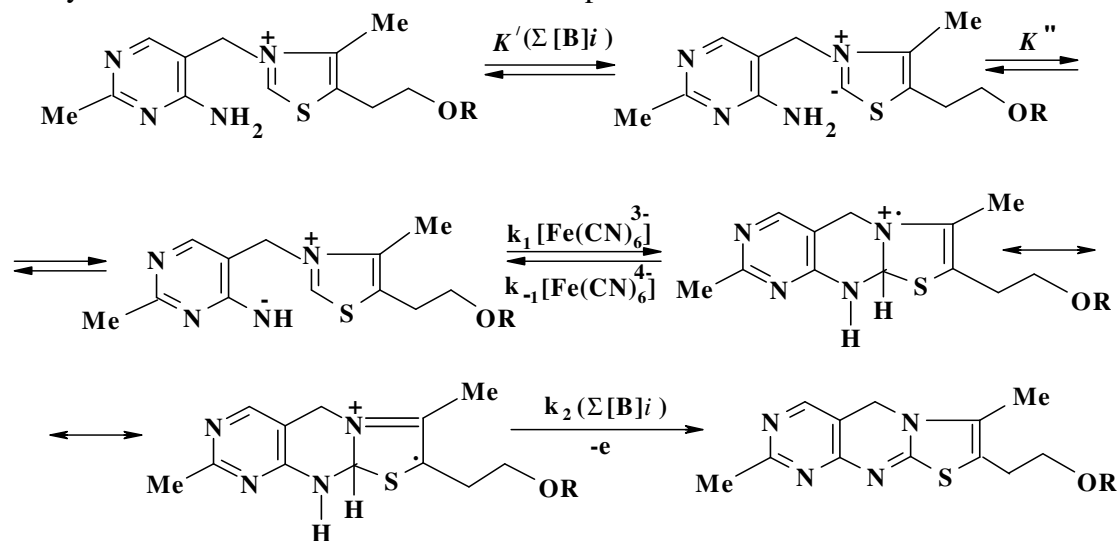
MECHANISTIC MODELS OF OXIDATIVE TRANSFORMATIONS OF THIAMIN AND THIAMIN PHOSPHATES

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Thiamin (vitamin B₁) is synthesized in the plant leaves and serves of essential metabolic function. The thiamine compound in plant tissue are present in form of unphosphorylated thiamin and in forms of thiamin phosphates. Thiamin diphosphate is the coenzyme of pyruvate dehydrogenase and α -ketoglutarate dehydrogenase complexes, transketolase and other carbohydrate enzymes. In plants, thiamin diphosphate catalyzes also conversion of pyruvate to acetolactate which is included in bisynthesis of branched-chain amino acid.

It is known that thiamin and thiamin phosphates during its functions in living cells in the presence of hydroxide may undergo oxidative transformations. We have studied the kinetics of model thiamin, thiamin monophosphate and thiamin diphosphate oxidation by ferricyanide to the thiochrome derivatives in phosphate buffer at pH 7.5-8.0. The reaction is inhibited by ferrocyanide. The dependence of reciprocal of the observed pseudo-first-order rate constants on ferrocyanide concentration at determined initial concentration of ferricyanide is linear. In presence of excess of ferrocyanide the reaction is first order in substrate and oxidant concentrations. Analysis of the kinetic data reveals that oxidation by ferricyanide involves one-electron transfer step with thiazolium radical cation formation.



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

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GROWTH REGULATION OF CEREAL ROOT ROT AGENT ISOLATES WITH EXOGENOUS PHYTOHORMONES

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

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

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

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

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

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

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

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

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

Results have testified the *Streptomyces avermitilis* UCM Ac-2179 capacity for synthesis auxins and cytokinins at the both media. Auxins were represented by indolilacetic acid (1105.14 and 2094.56 nano-g per 1g of dry biomass accordingly above mentioned media); cytokinins were represented by *iso*-pentyladenin (2182.04 and 837.82 nano-g/g), zeatin (759.67 and 168.87 nano-g/g), and zeatin-riboside (906.61 and 4228.36 nanog/g). The difference in quantitative production of phytohormones by streptomycete grown in the various media may be connected with existence of some precursors in the soy-bean medium. At the other hand, avercom also is rich in phytohormones (nano-g per ml): indolilacetic acid 216.61, *iso*-pentyladenin 427.68, zeatin 148.9, and zeatin-riboside 117.9.

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

ANTIOXIDANT POTENTIAL OF STABLE NITROXIDE RADICAL CLUSTERED BY A RESORCINARENE PLATFORM

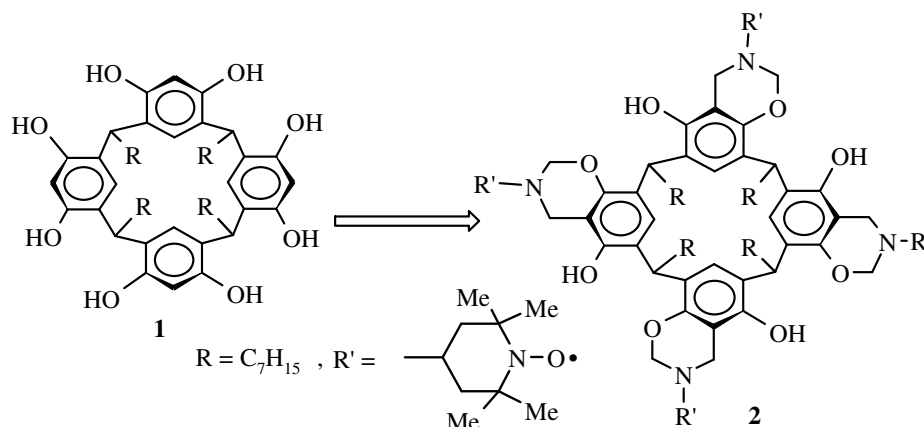
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Highly reactive oxygen species and lipid peroxy radicals are produced in living cells as a result of aerobic metabolism and are involved in a number of life sustaining biochemical processes. The failures of the antioxidant protecting systems initiated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



The radical scavenging abilities of C₄-symmetrical tetraoxazine derivative **2** were evaluated by effects on superoxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Superoxide mimic activity of spin labeled resorcinarene **2** was found to be may two more efficient compared to 4-hydroxy-TEMPO. DPPH scavenging ability of these macrocyclic compounds in term of stoichiometry is more than two orders of magnitude higher than for resorcinol. The results indicate that spin labeled resorcinarene **2** also effectively suppress the linoleic acid peroxidation in micelles in presence of free radical initiator. The screening test to determine the peroxy radical-trapping efficiency of compounds tested includes examine of influence of inhibitor on conjugated dienes formation from linoleic acid in micelles in presence of 2,2'-azobis(2-amidinopropane) as the initiator. Compound **2** was roughly an order of magnitude more active compared to Trolox C, known inhibitor of lipid peroxidation.

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

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

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

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

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

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

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

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

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

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

NEW CHEMICAL GROWTH FACTORS OF PLANTS

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

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

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

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

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

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

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

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

PERSPECTIVES OF GIBBERELLIN-PRODUCING PSEUDOMONAS RHIZOBACTERIA APPLICATION IN AGRICULTURE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The goal of our investigations was development of biotechnological methods of the microbial preparations intensifying with plant growth regulators (PGR). Objects of investigations were microorganisms selected in IMV NASU: *Bradyrhizobium japonicum* UCM B-6035 (basis of the preparation nitragine); *Bacillus megaterium* UCM B-5724 (basis of the preparation phosphobacterine); *Streptomyces avermitilis* UCM Ac -2177 producing avermectine complex (basis of the preparation avercom). Such PGR as emistime C, agrostimuline, eney and ivine, developed in the Institute of Bioorganic Chemistry and Petrochemistry of NASU and STC "Agrobiotech" were investigated.

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

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

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

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

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

METABOLIC ASPECTS OF METHYLOTROPHIC BACTERIA INTERACTION WITH PLANTS

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Aerobic methylotrophic bacteria (methylobacteria) and methanotrophs are widespread in nature and appear to be tightly connected with the plants. Earlier, such a connection was shown mainly for the pink-pigmented facultative methylobacteria (PPFM). Our studies on 140 species of mono- and dicotyledonous plants have shown that their seeds, phyllo- and rhizosphere are colonized by taxonomically different methylobacteria belonging to *Xanthobacter*, *Paracoccus*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, and also by methanotrophs of the *Methylocystis* genus. Remarkably, we found methylobacteria and methanotrophs on the surface and inside tissues that provides for these methylotrophs better survival at low and high temperatures or during drought. Alternatively, under favorable weather conditions followed by active plant growth and metabolism accompanied by emitting of volatile C₁-compounds, the methylotrophs form biofilms on the leaf surface, thus preventing the C₁-volatiles evaporation to the atmosphere.

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

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

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

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

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

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

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

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

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

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

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

In our researches we used the emistym C and reacom preparations as possible factors of optimization of plant growth on oil contaminated soils, which are characterized with deficiency of nutritive elements for plants. Emistym C is the product of endophytic fungi, it contains the balanced complex of phytohormons of cytokinine and hybereline nature, amino acids, sugars, peptides, unsaturated fatty acids and microelements. Reacom is a composition of chelate forms of microelements and balanced amount of the NPK active forms. We have analyzed the morphometrical indexes and measured of sum of chlorophylls ($a+b$) and their correlation in sedge plants under the action of oil contamination, emistym C (1×10^4 ; 2×10^4 ; 1×10^3) and reacom (dilution 5×10^4).

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

For phytoremediation of soils from oil contamination by a sedge plants large value has the state of underground part of plant – rhizome. It was shown, that plant root system for actions of reacom essentially changed. Rhizomes diameter and the amount of roots on them increased. Diameter of rhizomes of *Carex hirta* plants in apical part for reacom actions increased on 28 % in comparison with the control. Reacom positive influence on development of rhizomes was kept even when oil was added to the soil – diameter of rhizomes was 24 % much with respect to the controls. Emistym did not influence on the growth of rhizomes, diameter remained within the limits of the control. Diameter of rhizomes was 20 % lower than in the controls under compatible action of emistym and oil. The complex application of reacom, emistym and oil had a positive influence – diameter was 16% higher compared with the control and it did not differ significantly from the separate influence of reacom. Thus, for optimization of growth of sedge rhizomes in the conditions of oil contamination it was enough to use only reacom.

Level of chlorophyll sum ($a+b$) and their correlation in the leaves of *Carex hirta* plants under the action of oil contamination, reacom and emistym C were determined. The chlorophyll b content increased compared with the chlorophyll a content and that could be explained by adaptation of plastids of plants to the stress conditions. The decrease of green pigments concentration under complex influence of reacom, emistym and oil was observed. The content of chlorophyll did not differ significantly between controls and emistym C (dilution 1×10^3) treated plants. Such results were obtained in plants from oil polluted pots under the action of reacom. This confirms the inexpediency to connect reacom and emistym C for the treatment of plants which grow on oil contaminated soil.

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

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In cereal grains α -amylase carries out a key role in the starch mobilization during germination. Enzyme synthesis localized in two parts of grain, they are aleurone and scutellum, and then begin the process of its secretion to endosperm. The α -amylase synthesis induced with phytohormone GA₃, another hormone ABA carries out the opposite activity on this process.

In sprouting cereal grains there are more than ten individual α -amylase isoenzymes, which divided on two basic groups according their isoelectric points and some other biochemical properties and physiological destination. In the given work it was investigated the influence of GA₃ and ABA on the synthesis induction of different α -amylase isoenzymes in aleurone and scutellum of cereal grains.

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

Obtained data specify existence of the significant distinctions in α -amylase synthesis in embryo with scutellum and aleurone cells. Scutellum cells are poorly susceptible to the hormone signal, which is expressed in α -amylase activity change ability. In the case of embryo half seeds hormones addition did not lead to α -amylase activity change at least the first 72 hour of incubation. On the contrary deembryonated half seed showed high sensitivity to hormone action. GA₃ presence lead to sharp α -amylase activation already at the initial incubation stage, during 24-48 hours, ABA had braking effect on this process.

With the help of native electrophoresis was shown the different isoenzymes sensitivity on GA₃ and ABA action. On without embryo half seeds was shown that GA₃ induced the synthesis of anodic (group A) isoenzymes and repressed the activity of cathodic (group C) enzymes. Opposite action rendered for ABA at which presence synthesis of α -amylase group A was completely suppressed.

Summarising the above-stated and our research data it is possible to conclude that in cereal grains the synthesis of α -amylase isoform with low isoelectropoints (group A) is strictly adjusted by endohormones level (GA₃ and ABA). The mechanism of an high isoelectropoints (group C) possible is more combined and can include double control – phytohormones and sugarmetabolites action.

THE SILKPREPARATION EFFECT UPON CUCUMBER PLANT GROWTH IN HYPERTHERMAL CONDITIONS

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

NEW REGULATORY COMPOUNDS FOR PLANT GROWTH AND RESISTANCE

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Modification of the hormonal balance is a strong factor of regulation of the production process under unfavorable conditions. Therefore treatment of plants with natural or synthetic regulators of growth is widely used to increase plant resistance. It was demonstrated that treatment of plant leaves with methanol, a low-molecular one-carbon alcohol, caused a decrease in the negative impact of water deficiency on the production process of plants. It should be noted that methanol is a toxic agent, which makes it impossible to use this alcohol for practical purposes. Like methanol, some of its nontoxic analogues (LLB, PNBA, Fpeg, Fty, etc.) were also shown to have a positive effect on plants. By now some of these agents have been patented and adopted for practical use. However major effects of these agents on the general metabolism of plants should be studied in experiments with methanol, because this compound is simpler and more closely associated with plants than its analogues. It is very important to use methanol in model experiments, because it is produced in plant leaves and play a possible role in the enhancement of plant resistance. The goal of this work was to study the rate of CO₂ gas exchange, transpiration, stomatal resistance, and efficiency of the use of water in sugar beet leaves of different strata treated with methanol solution and to assess the stability of the photosynthetic apparatus against the background of increasing water deficiency in soil. The activities of some enzymes were also estimated.

Sugar beet (*Beta vulgaris*) plants were grown in controlled conditions with a temperature regime 30/30 °C (day/night), relative air humidity 70%, and 24-h light/dark photoperiod with a light intensity 1000-1200 μmol m⁻² s⁻¹. Measurements and methanol treatment were carried out using 40- to 65-day-old plants at the growth stage of 5–7 leaves. The plants were sprayed with a solution containing 40% methanol, 1 mM glycine (source of nitrogen) and 200 ppm detergent sylvet (pH 6.8). Control plants were treated with the same solution but without methanol. During the next 9 days of the experiment the volume of water used for watering each jar was gradually reduced. As a result the plants during the experiment were exposed to conditions with high intensity of light, enhanced temperature, and gradually increasing water deficiency in soil (from 0 to –100 kPa). The resistance to stress caused by water deficiency in soil was 1.3–1.9 times higher in the plants treated with methanol, than in the control plants. The methanol foliar spray eliminated the negative effect of water stress on photosynthesis, transpiration, and stomatal conductivity. Namely, under drought conditions, Rubisco activity, rate of CO₂ assimilation, stomatal conductivity and the rate of transpiration were higher in the methanol-treated plants as compare to control plants. Increase in malic enzyme activity indicating activation of anaplerotic way of carbon metabolism was also observed in the methanol-treated plants.

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

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

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

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

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

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Naturally plants during all life cycle influenced various factors of an environment. Special value ultra-violet radiation (UVR) (has 180-400 nanometers), a part of electromagnetic radiation of the Sun. According to forecasts, the future global changes of a climate connected with an exhaustion of an ozone cloud, entail increase in a doze of radiation getting on Earth UV. In this connection the knowledge of mechanisms of action UVR on physiological processes at plants, and especially on agricultural crops, gets the big theoretical and practical value. The primary goal of our research - to establish physiological effects of action UVR on growth, development and biological efficiency of a potato. Researches are executed on regenerants of potato (*Solanum tuberosum* L.) grades Odyssey and Yavor the Belarus selection, which grew up under lamps DNAZ-400 (the photoperiod – 16 hours) on artificial substrata at a room temperature. As source UVR mercury lamp DRT – 1000 served. For the control of size of a doze of an irradiation of plants used UVR – dosimeter DAU – 81. The unitary doze (E1) regenerants of potato made UV-irradiations 120 Dg/m². Variants of experience carried out all in 3-5 multiple frequencies.

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

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

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Known growth retardant - 2-chloroethyl-trimethylammonium chloride (CCC) and its analogue 2-ethyl-trimethylammonium chloride (CC) were shown to have influence on growth and greening of etiolated wheat seedlings. It is known that changes in balance of phytohormones play an important role in effects of growth retardants on plant growth and activity of the photosynthetic apparatus (PA). The aim of our research was to study the interrelationship between changes in levels of hormones and effects of choline compounds on growth and greening of wheat and bean seedlings. Photochemical activity of PS II was assessed by measurements of chlorophyll *a* variable and delayed fluorescence using phosphoroscope. The pigment contents were determined spectrophotometrically. The levels of abscisic acid (ABA) and cytokinins were assessed by enzyme-linked immunosorbent assay. The effects of CCC and CC at concentrations of 1 μM –5mM on growth, greening and formation of the PA in etiolated wheat seedlings were examined (1). A short-term root application of CC and CCC inhibited elongation of the coleoptile and first leaf but cholines accelerated these growth responses when the seedlings were exposed to white light. The first leaf appearance was accelerated as well. Effects of cholines were observed during 96 h of light exposure after the pre-treatment of 4-d-old seedlings with cholines and depended on the type and concentration of compound. CCC and CC accelerated greening and increased the photochemical activity of PS II in seedlings. Stimulation of greening by cholines was accompanied by accelerated accumulation of cytokinin-like substances (2) detected in the first leaves. Taking into account that root application of kinetin (10^{-4} - 10^{-6} M) stimulated greening we concluded that the influence of cholines on concentration of substances with cytokinin activity detected in leaves might be involved in the stimulation of Chl (*a+b*) accumulation. Root application of GA₃ (10^{-4} - 10^{-6} M) lead to partial decline in inhibitory effect of CCC on leaf and coleoptile growth. Besides, pretreatment with CCC led to increase in level of ABA and decline in content of gibberellins in primary leaves of bean (*Phaseolus vulgaris* L.) seedlings. Thus, inhibitory effects of choline compounds on growth may be due to enhanced formation of ABA and reduced level of gibberellins resulting in choline pretreatment.

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

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

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

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

The following organic acids were used as the growth stimulators : citric $\text{CH}_2\text{C}(\text{OH})\text{CH}_2(\text{COOH})_3$, α -ketoglutaric $\text{HOCCOCH}_2\text{CH}_2\text{COOH}$, succinic $\text{C}_2\text{H}_4(\text{COOH})_2$, malic $\text{HOCC}(\text{OH})\text{CH}_2\text{COOH}$, oxalic $(\text{COOH})_2$. The solutions with different molar relationship of acids were prepared, namely, solution №1 (1:1:1:1:1), №2 (1:2:3:4:5), №3 (5:4:3:2:1).

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

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

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

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

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

SECONDARY HORMONE OF CYTOKININ AND 14-3-3 PROTEINS ACTIVATE Ca²⁺-DEPENDENT ATP-ASE OF PLASMATIC MEMBRANE FROM ALEURON LAYER OF WHEAT SEEDS

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

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

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

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

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

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

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

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

The investigation of properties of activated ATP-ase of plasmatic membrane of aleuron layer shows that this enzyme related to Ca²⁺ dependent ATP-ase.

Namely this Ca- ATP-ase participates in increasing of the level of cytosolic Ca²⁺ in the cells of higher plants. It was very surprisingly that CSH and cell free extracts from wheat embryos and CSH and cell-free extracts from sheep brain causes the activation of ATP-ase of plasmatic membrane of aleurone layer only with ions but not with Mg²⁺ ions.

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

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

USING OF ETHYLENE AND FATTY ACIDS AS MARKERS OF WHEAT VARIETIES

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

BIOPREPARATION "MYCOLIN" AND ITS EFFECT ON BARLEY PLANTS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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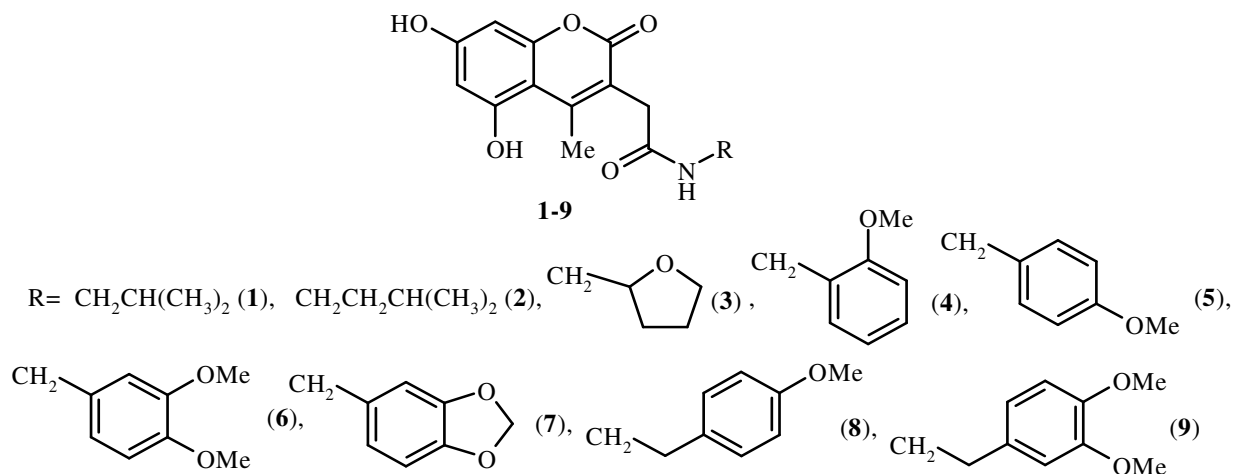
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The mechanism of pathogenesis induced by herbicides inhibitors of acetyl-CoA carboxylase (ACC) was investigated. Effect of herbicide haloxyfop-R-methyl (HRM) on lipids peroxidation (POL) reactions and on dynamics of necrosis induction in meristematic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

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

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



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

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

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

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

STIMULATORS AND INHIBITORS OF GROWTH OF ALDER SPECIES UNDER PERMAFROST CONDITIONS

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

Plants of *D. fruticosa* and *A. hirsuta* were studied during the summer-autumn seasons of 2006 in the vicinity of Yakutsk (62⁰ N, 129⁰ E). Each examined plant of both species had nodulation of a coral-like shape. We found to 25 g of nodules in dry weight near trunk area in the ring of 0.5 m in diameter. The root system was removed from soil and rinsed, nodules removed either, then dried and weighed. The ether fraction of the examined organs of plants was isolated by applying a thin layer chromatography method – isopropanol-ammonia-water solvent (10:1:1) – with further subdivision into ten zones to determine biological activity in each zone by means of biotesting for the gain of segments of wheat coleoptiles. 15% of stimulating or inhibition was taken as a confidence level. We did not determine nitrogen-fixing activity of nodules but it has been known that it is commonly in direct proportion to their amount and weight.

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

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

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

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

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

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

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

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

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

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

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

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

BIOLOGICAL ACTIVITY OF ENDOPHYTIC BACTERIA IN THE GENUS *METHYLOBACTERIUM*

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

UKRANIAN PLANT GROWTH REGULATORS FROM IDEA TO REALITY

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

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

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

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

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

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

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

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Maize is valuable agricultural crop owing to food and fodder qualities. It is possessed of malate type C₄-photosynthesis, drought-stability, high productive and so it's growing is vindicated in the South of Ukraine. At the same time it has lower salt-stability (0,6% soil salinity is critical) and thus stipulates for problem of it's growing on weak salinitive soils, which area are culminated 30% in Ukraine. In maize individual development mark out 9 men phases and 12 – women gametophytes, each of which is characterized specifical anatomical-morphological and physiological-biochemical peculiarities, calling forth differential perceptibility to different environment factors in ontogenesis.

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

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

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

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

*Numerator – a quantity of plants and ears, which are remained from variants (10 reiteration in each);

**denominator – mean value of proper parameter.

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

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

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

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

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

The primary results and progress are summarized as follows:

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

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

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Arabinogalactan proteins (AGPs) are a superfamily of structurally complex proteoglycans, implicated in various aspects of plant growth and development. AGPs are particularly abundant at the outer face of plasma membrane, in secretion and mucilages and in conditioned medium of *in vitro* cultured cells. “Classical” AGPs containing glycosylphosphatidylinositol plasma membrane anchor can interact with cell wall polymers making dynamic adhesion zones and function to link the PM to the cytoskeleton. Previous studies have shown that morphogenic calli of tartar buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) has produced up to 30-100 – fold more extracellular AGPs as compared to non-morphogenic calli. It was proposed that such difference in AGP secretion seem to be conditioned by essential role of AGPs in keeping of morphogenic state of callus. Here, we show that the addition of (β -D-glucosyl)₃ Yariv reagent (which selectively binds and perturbs AGPs) to the medium of callus-cultured cells in concentration of 250 μ M do not change the growth activity of non-morphogenic callus. In contrast, in morphogenic callus, the same Yariv reagent treatment results (in dependence on 2,4-D in culture medium): 1- in 50% inhibition of proembryonal cell complex (PECCs) formation (medium with 2,4-D); 2- in completely inhibition of bud and embryoid formation but preservation, at the same time, of root formation (medium without 2,4-D). Since PECCs, as well as buds and embryoids are originated from cells of surface layers of callus (i.e. exogenously), while roots are formed endogenously, we proposed that Yariv reagent has broken the structural integrity of surface cells, thereby preventing their further differentiation or even inducing cell death. Histologically, treated by reagent Yariv callus clumps showed a “bulging” phenotype of surficial cells. On transmission electron microscopy level, we revealed that Yariv reagent treatment resulted in hard deposition of electron dense material on cell wall, plasma membrane, and throughout intercellular spaces of surface cells. In almost all surficial and, in lesser degree, in subsurficial cells, it was shown the more or less prominent exfoliation of plasma membrane from cell wall. In certain cells, however, we revealed also a plasma membrane ruptures. Cytoplasm shrinkage and formation of apoptotic-like packets was seen mainly in bulging cells. It is known that plant polarity and morphogenesis is controlled via integral coordination of the cytoskeleton and the cell wall functions. By the means of our results we can propose that the disruption of AGPs at the cell wall – plasma membrane interface, which tends to loss of adhesion contacts, will cause the disturbance or loss signal transduction and, finally, change cell viability, proliferation and morphogenic response of cultured cells.

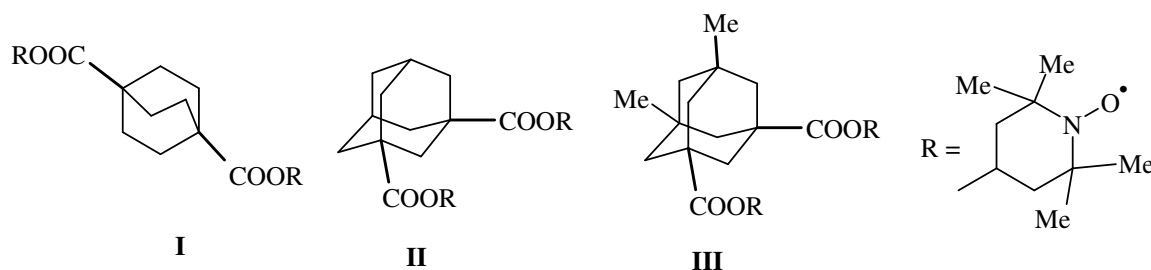
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BINDING OF NITROXYL BIRADICALS TO THE THYLAKOID MEMBRANES

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



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

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

THE REACTION OF WINTER WHEAT PHITOHORMONE SYSTEM ON MINERAL NUTRITION CHANGE

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

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

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

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

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

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

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

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

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

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

ANTISTRESSFUL ACTION OF JASMONIC ACID CHEMICAL ANALOGUES ON WHEAT PLANTS

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

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

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

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ABOUT THE MECHANISMS OF PLANT GROWTH REGULATOR ACTION AT A GENETIC

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

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

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

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

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

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

$$K_{Eff}^i = \left(\frac{Par_i}{Par_k} - 1 \right) \times 100\%, \text{ where } Par_i \text{ is a value of vital functions parameter of}$$

microalgae population in measuring, and Par_k is a value of this parameter in a control test.

BA of examined preparation is determined from expression

$$K_{BA}^i = K_{Eff}^i / C, \text{ where } C \text{ - is a concentration of examined matter, } \%$$

It was shown that at the estimation of biological effect of the probed preparations in the range of concentrations from $0,5 \times 10^{-6} \%$ to $2 \times 10^{-5} \%$ substantial changes in the coefficients of

biological efficiency were not observed. Biological Effect of *Emistim C* with during the concentration of $0,5 \times 10^{-6} \%$ $K_{Eff}^{Bm} = 24,7 \%$, and at $2 \times 10^{-5} \%$ $K_{Eff}^{Bm} = 22,6\%$. However,

at the analysis of *Emistim C* BA coefficients, the substantial diminishing in this range of concentrations - from $4,95 \times 10^6$ to $11,32 \times 10^5$, i.e. K_{BA}^{Bm} was diminished more, than in 4

times. Similar results were got and at an analysis K_{BA}^i on a parameter “energy of population”. In this case at the increase of operating matter concentration there was diminishing in two times – from $3,19 \times 10^6$ to $16,65 \times 10^5$.

Analogous researches were conducted on plant growth regulators *Ivin*. A range of the probed concentrations was from $2,5 \times 10^{-5} \%$ to $5 \times 10^{-4} \%$. K_{Eff} changed in limits from 10,4% to

33,96%. Thus also there was falling of BA with the increase of operating matter concentration. Maximal K_{Eff} of *Ivin* looked after during the concentration of 2×10^{-4} , for

which he was equal $33,6 \times 10^4$. During the concentration of $5 \times 10^{-4} \%$ $K_{BA} = 13,6 \times 10^3$, i.e.

diminished more, than in twenty times.

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

ENDOGENOUS PHENOL COMPOUNDS AND OIL-CONTENT OF COTTON SEEDS

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

The important component is presence of activators and inhibitors adjusting a metabolism, defining specific specificity and efficiency of plants. In this connection, a cotton-plant with presence of endogenous inhibitor of reaction – gossypol (2,2-di, 1,6,7-trioxy-3-methyl-5-isopropyl-8-aldehydonaphthyl) presents special interest. Gossypol – specific for *Gossypium* type compound of polyphenol nature. It is being formed from the first days of cotton growth and is present in all vegetative and generative bodies. As the molecule has numerous functional groups (hydroxyl, aldehyde), depending on environment where the reaction takes place, it can be an acceptor and the donor of electrons. Gossypol has rather high oxidation-reduction potential ($B_0=0,77$) close to oxygen potential ($B_0=0,82$) and participates in cell respiration processes as one of key interim electron carrier.

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

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

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

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

HORMONAL REGULATION OF PLANT RESPONSE TO WOUNDING

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