SESSION 3:
Brassinosteroid signaling

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

SYNTHESIS AND STUDY OF NOVEL OF BRASSINOSTEROID DERIVATIVES

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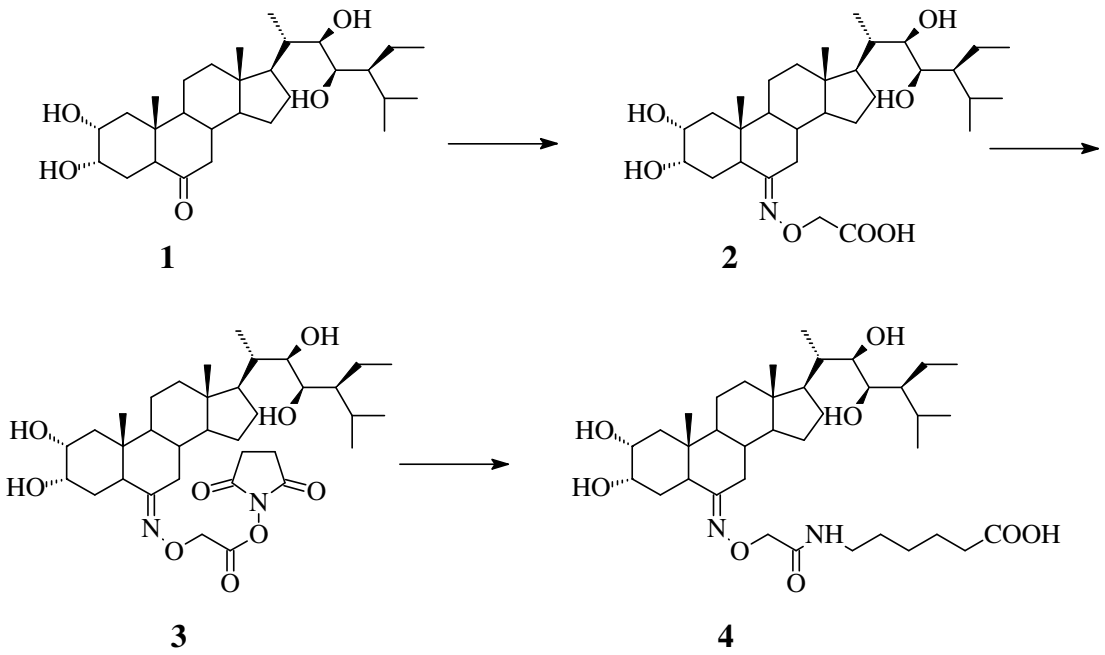
Brassinosteriods (BS), a new group of phytohormones, are plant growth regulators and adaptogens[1]. In the course of our work on BS synthesis we prepared novel 6-oxime derivatives of 28-homobrassinosteroid. The effective synthesis of the title compounds based on the reaction of 28-homocastasterone **1**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

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



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

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

BRASSINOSTEROIDS, RESISTANCE AND PRODUCTIVITY OF WHEAT

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

COMPOSITE PROTECTIVE SUBSTANCES OF NATURAL ORIGIN

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

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

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

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

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