Features of the biochemical adaptation of agricultural crops at the action of the biotic and abiotic factors of environment

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Abstract
Effects of Fusarium spp., Bipolaris spp., drought and salicylic acid (SA) on the modification of protein and enzymic complex, oxiding and antioxiding processes, the content of endogenous SA in the winter wheat, spring barley and maize plants that differ in their resistance to pathogens and drought have been studied.
Changes in cell walls lectin activity and in the carbohydrate specificity of lectins induced by SA or a pathogen in plants from different families of cereals have been demonstrated. A level of wheat, barley and maize genotypes resistance to Fusarium spp., Bipolaris spp. affected to the rate of these changes. It was supposed that not only generic but also genetics differences in the specificity of the lectins’ accumulation and redistribution lie in the basis of differential sympathy and, as investigated, efficiency of participation of these proteins in the adaptive reactions.
It was shown that the changes of dynamic of lipoxygenase activity (LOX), phenyalanine ammonia-lyase activity (PAL) in the cereals genotypes at infection of Fusarium spp., Bipolaris spp. and at the action of SA have a different orientation in junction with a degree of genotype resistance and culture genus that testifies to adaptation character of these changes. Functions and role of SA, H2O2 and NO in the seedlings of cereal crops at pathogenesis were explored. The differentiated changes of these indicators are founded. They were connected with the level of the genotypes resistance of wheat, barley and maize to Fusarium spp., Bipolaris spp.

Key words: wheat, barley, maize, resistance, salicylic acid, drought, Fusarium spp., Bipolaris spp.

Introduction
Investigation of plant adaptation to unfavorable environmental factors was always important and directed to the solution of a problem obtaining high yield of agricultural plants. Creating genotypes of adaptive resistance plants assume resistance to the complex of all adverse factors of environment. Formation of plant resistance at stressful situations is connected to the set of protective mechanisms which responsible for preservation of plant viability and metabolism reorganization (Dixon et al, 1994: Hammond-Kosach et al, 1996). The main role in the adaptation to influence of environment adverse factors belongs to biochemical systems.

It was established that decline of a lipid peroxidation (LPO) with the safety of antioxidants level (catalase activity, peroxidase activity, glutathione content) of seedlings in maize genotypes are plant adaptive reactions at a drought. It was showed that strengthening of LPO with a next mobilization of antioxidants level could serve as one of the protective reactions of cereal crops at infection by Fusarium spp., Bipolaris spp.

It was concluded that studied physiological-biochemical processes take part in formatting mechanisms of cereals crops resistance to Fusarium spp., Bipolaris spp. at drought; and SA is an activator of immune properties of cereal crops. Based on the received results of estimation of wheat and barley genotype resistance to Fusarium spp., Bipolaris spp. new methods were developed. It was got two Ukrainian patents for this methods (patent #12639, declarative Ukrainian patent #43280).
various signal systems activation (Tarchevsky, 2002). Primary elicitor signals have various natures and one of elicitors is SA (Raskin, 1992). It is supposed that starting of stress reaction triggers by displacement of prooxidation-oxidation balance to the direction of lipid peroxidation activating. Thus products of the LPO can be both “indicators” and “primary mediators” of a stress which is a special state of a cell and can lead to its resistance increasing (Baraboy, 1992). At the same time mechanisms of plant protection activation of biochemical systems by stress are not quite clear in many respects. In particular, a question about nature of intermediates between stress influences and cell’s biochemical answer is open till now.

In this work we studied effecting of exogenous SA, pathogens and drought to changes in lectins, LOX and PAL activities; to accumulation of H2O2, NO, SA and to activation of LPO of winter wheat, spring barley, maize cultivars that differ in their resistance to Fusarium spp., Bipolaris spp. and a drought. An additional purpose of this work was a determination whether these metabolic active substances can have specific functions in the mechanisms of activation of protective biochemical systems at biotic and abiotic stresses.

Materials and Methods.
The objects of this study were 3 – 10-days seedling of the 50 cultivars of winter wheat (Triticum aestivum L.), spring barley and seedlings of the 10 line of maize (Zea mays) that differ in their resistance to Fusarium spp., Bipolaris spp. and drought and on four methods of germination (in distilled water (reference), in presence of 2 mM SA, in presence of pathogens and at water deficit). The source of the infection was a pathogenetic strain of the Fusarium graminearum (for winter wheat), Fusarium culmorum, Bipolaris sorokiniana (for spring barley), Fusarium moniliforme (for maize) in a concentration of 10^5 conidia/ml of liquid potato medium. Lectin activity was studied by the method of hemagglutination response of white rats trypsinised erythrocytes (Malichenko et al, 1994). Reaction of lectin competitive inhibition carried out by method of Lutsik (Lutsik, 1981). Activity of LOX was measured by absorption at 440 nm using linoleic acid as a substrate by the method of Budnichkaya (Budnichkaya,1955). Activity of PAL was determined by modified method of Zucker (Zucker,1965). SA concentration was quantified by HPLC (Raskin et al, 1989). Lipid peroxidation was measured by thiobarbiturate method for the malonedialdehyde thiobarbiturate adduct (MDA) accumulation spectrophotometrically (Uchida et al, 1999). Glutathione content is determined using Ellman reagent (Grishko et al, 2002). A catalase activity was determined by method of Koroljuk (Koroljuk et al, 1988). H2O2 was measured by the fluorometric method (Ebermann et al, 1987). NO content was measured by determination of the levels of nitric oxide stabile metabolites: NO2⁻ and NO3⁻ (Komarevtseva et al, 2002).

Biological experiments were carried out in triplicates and all other analyses in duplicates or triplicates.

Results and discussion
The strategy of a plant at both biotic and abiotic stresses consist in response to these action by activation some protective reactions. It is shown that each kind of stresses has both general and specific defense mechanisms.

One of the earliest reactions of a plant cell to stress is an activation of plant lectins. A basis of their biological activity is a participation in the carbohydrate-protein interactions and is characterized their selection to various carbohydrate determinants. Therefore change of the carbohydrate specificity at the different influences is used for the qualitative characteristic of these proteins (Hayrullin et al, 1993). Our studying of changes in the cell wall lectins activity and in the lectins’ carbohydrate specificity in plants from different families of cereals in a presence of pathogens and under the action of 2 mM SA has allowed concluding that level of wheat, barley and maize genotype resistance to Fusarium spp., Bipolaris spp. affects to rate of these changes (see table).
It is supposed that metabolites forming under the action of a SA and pathogens and acting as a signal molecules transmitting a signal to the cell genetics system, probably, connected to different potentials of energy and plastic resources for synthesis of intermediary substances regulating an induction of lectin activity. It was assumed not only generic but also genetics differences in the specificity of the lectins accumulation and redistribution lie in the basis of differential sympathy and (as was investigated) efficiency of participation of these proteins in the adaptive reactions.

Electrophoretic protein spectrum of lectins of the seedlings of winter wheat differing on resistance to *Fusarium spp.* was studied. Our assumption was confirmed that changes of lectin activity and its carbohydrate specificity at action of the pathogen and 2 mM SA are connected with change of their componential composition and appearance of a new component with M.w. 27 kD. Evidently, lectins formation with various carbohydrate specificities possessing is activating under the action of a stress. It is demonstrated under action of a stress the intensity of polypeptide zones in resistant genotypes was more higher compare of susceptible ones.

This time is noted that synthesis of many enzymes including LOX and PAL is inducing by the stress. It is shown that stimulation of LOX and fast LPO considers as the general answer of the plants at biotic and abiotic stresses. LOX-activated oxidation of linolenic acid leads to synthesis of jasmonic acid which being a signal molecule causing activation of many plant protective reactions (Hildebrand et al, 1987). For understanding a possible role of LOX in the plant responses at the pathogenesis and in the presence of 2 mM salicylic acid we watched dynamics of LOX activity changing in the shoots and roots of the seedlings of various cereal crops cultivars which differ on resistance to *Fusarium spp.*, *Bipolaris spp.* Summarize our results we have noted that dynamic of LOX-activity change under *Fusarium spp.*, *Bipolaris spp.* and 2 mM SA in germination process of the different families of cereal crops differs them by resistance level. A reverse correlation between genotype resistance to pathogen and total LOX activity in the tissues of an elevated part of resistant genotypes of winter wheat and spring barley seedlings and the right correlation in the seedlings of maize were observed. At the same time in tissues of roots right correlation was marked in the seedlings of winter wheat and spring barley; this dependence in the tissues of the maize roots has negative character (see table).

It is known that infectious process accompanies with accumulation and synthesis of many proteins, phenols; formation of lignin and some other chemical substances (Legrand, 1983). Amplification of the free phenols synthesis and lignification correlates in most cases with increasing key enzyme of phenolic metabolism – PAL. PAL participates in formation of predecessors of SA, phytoalexins and monomers of lignin, changing mechanical and chemical barriers of plant cells (Fellegrini, 1994).

Studying total induction of PAL in the tissues of elevated part and roots of seedlings on the background of 2 mM SA and pathogen has confirmed our earlier assumption about unequal sensitivity of enzymes to these factors in different genotypes of cereal crops (see table). Most likely, metabolites forming by their action have various nature and action mechanism and, as consequence, possesses by different opportunities in activation of the construction and energy processes.

By analyzing received results it is definitely possible to tell a following: PAL participates in plant responses at the action of 2 mM SA and *Fusarium spp.*, *Bipolaris spp.* Level of the total PAL activity changes in elevated part’s tissues of seedlings; in some cases it precisely correlates with plant resistance, but at the same time this rule is not a general. However it’s possible that PAL is a basic component of those control mechanism which functioning determines sizes of the quantitative shifts in accumulating of phenolic substances and intensifications of a cell lignification processes which, as known, participating in protective responses formation.

A role of active oxygen species (AOS) in induction of plant protective answer is interesting and inconsistent. It is marked that a primary product of restoration of molecular oxygen is superoxide anion $O_2^-$ which turns in hydroxide radical $HO_2^-$ and then in $H_2O_2$. $H_2O_2$ possesses very high stability in the water solutions and has, depending on conditions, as oxidizing as well
regenerative properties. H$_2$O$_2$ can render a direct antimicrobial effect; catalyze the mechanical enforcing of the cellular walls. It is the secondary intermediary in SA-dependent signal system (Lamb et al, 1997).

It was shown by our researches that H$_2$O$_2$ level in resistant genotypes of cereal crops at the action of 2 mM SA and pathogens was higher compare to susceptible ones. Accumulation of AOS at pathogenesis and other stressors induces a LPO. Toxic products of LPO can carry out a quick answer of a plant at the first stages of pathogenesis. Remote stages of protection are carried out by the oxidizing enzymes connecting with the formation of oxygen radicals – lipoxygenase, peroxidase. Oxidizing processes in a cell are limited by endocellular mechanisms of the antioxidant protection. Antioxidants of direct action are superoxide dismutase, catalase, peroxidase, glutathione (Taran et al, 2004).

In our researches we have noted certain specificity in changing of intensity of LPO and contents of glutathione in various sorts of cereal crops at the infection and at the action of SA in the dependence of genotype resistance to diseases (Table).

It is known that the salicylic-sensitive and salicylic-tolerant forms of catalase that coding by independent genes presents in the plants. It is established that catalase is a SA-connecting protein which activity is blocking by SA that leads to H$_2$O$_2$ accumulation. Forming H$_2$O$_2$ or other kinds of AOS activates expression of the protective genes (Rao et al, 1997).

Studying a change of catalase activity dynamics in the investigated cereal crops’ seedlings at the pathogenesis and the action of a SA has allowed revealing features in change of activity of given enzyme at various cultures. The level of catalase activity was higher in the resistant genotypes compare to susceptible genotypes in seedlings of spring barley and maize at the infection by *Fusarium spp.*, *Bipolaris spp.*; both in the elevated part of seedlings and in their roots. The inverse relationship in changing of catalase activity was observed in the seedlings of winter wheat. Studying of catalase activity on background of SA has allowed to differentiate enzymes of cereal crops to salicylic-sensitive and salicylic-tolerant. So, a reduction of catalase activity both at resistant genotypes and at susceptible ones in seedlings of spring barley at the influence of exogenous SA was observed. At the same time SA caused increase of catalase activity in seedlings of maize resistant lines. Similar dependence was observed in the susceptible genotypes of winter wheat. Such various changes of catalase activity were caused at the given influences; most likely by distinctions in functioning of antioxidizing systems of a cell in formation of AOS and difference in sensitivity to SA catalase forms in cereal crops genotypes various on resistance to phytodiseases (see table).

Some other changes of catalase and peroxidase activities and glutathione and products of LPO levels were showed at water deficit in maize lines which differ on resistance to drought (see Figure). A decreasing in the intensity of LPO and catalase activity with increasing in a level of glutathione and keeping of peroxidase activity at the level of control plants was typical at drought for resistant lines of maize. In susceptible lines of maize action of a drought caused increase the activation of LPO, catalase activity, peroxidase activity and reduction of concentration of glutathione. Most likely distinctions in a level of LPO in lines of maize differ in resistance to drought are connected with distinctions in working their antioxidant systems (see Figure).

It is established that complex and coordinated signal system which adjusts activation of plant protective reactions exists in plant cells (Dmitriev, 2003). The SA occupies an important place in plant signal systems. Its content grows at pathogenesis in plants (Delaney, 1994). At the same time it is proposed that SA is not the signal notifying a plant about danger but, however, it is necessary for signal transfer (Yu, 1997). It is shown by our laboratory’s researches that plants of cereal crops at infection and at action of exogenous SA change contents of free SA and conjugated forms of SA. Thus a level of endogenous SA at the infection is higher in the resistant genotypes compare to susceptible ones. At the action of the exogenous SA contents of the endogenous SA in plants rises irrespective of genotypes’ resistance to pathogens.
Existing schemes of SA action mechanism show a connection between SA and “oxidizing explosion”; one of its components is NO which increasing action of AOS and SA (Delledonne, 1998). Dynamic of NO-content changing at the infection of pathogens and influence of 2 mM SA has specificity in different genotypes of cereal crops depending on resistance to *Fusarium spp.*, *Bipolaris spp.* (see table).

The received results show that plants have deeply echeloned biochemical protection system against stress influences. Separate components of this system replace each other consequently, supplementing and increasing each other. Probably protective reactions to the action of pathogens and exogenous SA in cereal crops genotypes with different resistance have different mechanisms. It specifies by different level of all physiological-biochemical parameters of the metabolism which connect with formation of protective answer of a plant cell to stress in different resistance of genotypes. The received data are an acknowledgement of the assumptions which was made early by us on participation of the investigated biochemical factors in formation of defense mechanisms of cereal crops to *Fusarium spp.*, *Bipolaris spp.* and to drought. Methods of prognosis and estimation of resistant cereal crops genotypes to *Fusarium spp.* and *Bipolaris spp.*, as well as methods for control of agricultural product safety were developed based on these received results. Also for these methods we have got Ukrainian patents (No. 12639, 1997; No. 43280, 2001).

References.


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Table. Changes of biochemical parameters in shoots and roots of winter wheat, spring barley and maize seedlings, induced by salicylic acid and Fusarium spp.

| Biochemical Parameters | Winter wheat | | | Spring barley | | | Maize | | |
|------------------------|--------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                        | 2 mM SA      | Fusarium         | 2 mM SA          | Fusarium         | 2 mM SA          | Fusarium         | 2 mM SA          | Fusarium         |                  |                  |                  |
|                        | shoots       | roots            | shoots           | roots            | shoots           | roots            | shoots           | roots            |                  |                  |                  |
| **RESISTANT GENOTYPES**|              |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
| Lectin activity        | 913.8        | 247.4            | 63.8             | 394.9            | 208.7            | 483.8            | 248.2            | 119.4            | 220.6            | 86.1             |                  |
| LOX                    | 113.5        | 210.8            | 180.7            | 296.8            | 213.0            | 150.6            | 200.0            | 129.5            | 186.0            | 110.6            |                  |
| PAL                    | 132.8        | 103.7            | 133.6            | 227.0            | 100.0            | 119.0            | 100.0            | 109.0            | 72.0             | 130.0            |                  |
| LPO                    | 117.5        | 129.0            | 142.5            | 150.0            | 112.0            | 150.0            | 120.0            | 150.0            | 91.0             | 127.6            | 114.7            | 93.4             |
| Glutathione            | 94.7         | 106.5            | 86.3             | 104.1            | 99.1             | 151.9            | 54.9             | 63.5             | 70.0             | 75.1             |                  |
| Catalase               | 85.0         | 131.5            | 147.5            | 195.0            | 198.0            | 138.0            | 98.0             | 120.0            | 322.0            |                  |                  |
| Free SA                | 283.3        | 313.1            | 147.5            | 250.0            | 250.0            | 198.0            | 240.0            | 150.0            | 220.0            |                  |                  |
| Conjugated SA          | 250.5        | 385.0            | 207.0            | 350.0            | 350.0            | 220.0            | 154.6            | 170.0            | 392.6            |                  |                  |
| NO                     | 136.0        | 85.0             | 125.0            | 230.4            | 103.0            | 107.0            | 53.0             | 135.0            | 59.0             | 217.0            |                  |

| **SUSCEPTIBLE GENOTYPES**|              |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
| Lectin Activity        | 556.7        | 880.4            | 60.3             | 147.5            | 157.8            | 301.0            | 588.2            | 232.5            | 36.9             | 304.4            |                  |
| LOX                    | 230.8        | 86.3             | 147.5            | 269.2            | 529.6            | 81.5             | 66.7             | 272.4            | 57.9             | 168.9            |                  |
| PAL                    | 67.6         | 80.0             | 50.3             | 99.1             | 54.9             | 63.5             | 59.8             | 70.0             | 75.1             |                  |                  |
| LPO                    | 73.6         | 86.0             | 50.3             | 50.3             | 77.0             | 68.3             | 151.0            | 133.0            | 225.0            | 143.0            |                  |
| Glutathione            | 72.7         | 96.6             | 66.6             | 100.0            | 114.0            | 83.0             | 69.0             | 53.0             | 95.0             | 115.0            |                  |
| Catalase               | 160.0        | 130.0            | 170.0            | 87.0             | 65.0             | 70.0             | 87.0             | 75.0             | 47.0             | 82.0             |                  |
| Free SA                | 154.7        | 58.9             | 48.3             | 178.0            | 89.0             | 47.0             | 105.8            | 101.0            | 42.0             | 68.2             |                  |
| Conjugated SA          | 110.0        | 56.8             | 37.8             | 250.0            | 54.0             | 26.0             | 130.5            | 255.0            | 46.6             | 40.4             |                  |
| NO                     | 107.0        | 88.0             | 84.5             | 88.5             | 163.0            | 391.0            | 113.0            | 59.5             | 84.0             | 55.3             |                  |
CHANCE OF THE LEVEL OF LPO, GLUTATHIONE, CATALASE ACTIVITY AND PEROXIDASE ACTIVITY IN THE SEEDLINGS OF THE MAIZE AT THE DROUGHT