SUMMARY

Progressive climate changes create favorable conditions for the development of fungi, whose metabolites are toxic to both plant cells and humans. One from mycotoxins, produced by fungi of the genus *Fusarium*, accumulating in cells, is zearalenone (ZEA).

This substance stimulates the occurrence of toxic reactions, the effect of which is a reduction in the amount and quality of crops, agronomically important. Understanding the mechanism of stressful action of the ZEA may contribute to the development of methods minimizing the effects of this mycotoxin.

This issue is particularly important for plants that are the main raw material in the food industry, such as, for example, wheat. Occurring within species, varieties with greater or lesser sensitivity to stress conditions, can be research objects to search for differences/ similarities in the mechanisms that drive stressful/protective reactions.

The aim of the experiments was to investigate the differences in the stages of the stressful mechanism of ZEA action on tolerant and sensitive wheat cells, by determining the biochemical and physicochemical parameters that determine the intensity of stress.

Brassinosteroid (24-epibrassinolide, EBR), whose protective properties have been demonstrated in the presence of other stressors, has been tested as a potential protection factor in relation to ZEA. The biological object were the cells of spring wheat calli (*Triticum aevestum* L.), obtained from immature germs, which were grown for 3 days on medium containing ZEA at stressful (30 μM) or/and EBR, at hormonal concentration (0,1 μM). Control cells were growing on nutrients not supplemented by these substances Stimulation of oxidative stress (ROS generation) was also tested in parallel experiments in which the direct production of reactive oxygen species was obtained by cell ozonation.

In biochemical tests high performance liquid chromatograph (HPLC) coupled with mass spectrometry detector was used - to determine the accumulation of ZEA, whereas ultra-high performance liquid chromatograph (UHPLC) with quadrupole mass spectrometer detector - to determine the EBR content in cells.

Analyzes of enzymatic (SOD, CAT, POD) and non-enzymatic (AA, GSH, SA) antioxidants and osmoprotective substances (proline and enzymes mediating its synthesis) were made using spectrophotometers (UV and UV/VIS) equipped with monitoring of reaction kinetics. Membrane lipids extracted from callus cells were separated by column chromatography and the purity of the fractions was evaluated by thin-layer chromatography.

Structural - mechanical properties of layers formed from selected lipid fractions (phospholipid (PL) and galactolipid (MGDG)) were established on the basis of the physicochemical parameters determined using Langmuir technique.

On the basis of experiments it was found that in the conditions of stress caused by the presence of ZEA in the media, cultures of the sensitive variety calli were characterized by a smaller increase in fresh mass, while peroxidation of membrane lipids was larger than in the tolerant variety.

Activation of enzymatic and non-enzymatic antioxidants in the sensitive variety was mainly related to CAT enzymes and to synthesis of glutathione and proline, while the tolerance variety showed a significant increase in the activity of mainly SOD and POD enzymes, as well as an increase in the synthesis of ascorbic acid (as compared to the sensitive one). The higher proline content found in the sensitive variety arised from a shift in the metabolic pathway of this osmoprotectant, towards synthesis (via the P5CS enzyme), relative to the decomposition (by PDH) than was demonstrated in the tolerant variety.

The presence of EBR in the media containing ZEA resulted in an increase in mass increment and in a reduction in cell lipid peroxidation, compared to objects growing on media with ZEA, especially at the sensitive variety. The sensitive variety accumulated larger amounts of ZEA than tolerant, unlike EBR, that was absorbed at higher levels by the tolerant variety.

For both varieties growing on medium containing ZEA additional exposure to reactive oxygen species (ROS) led to an increased lipid peroxidation, increased CAT activity and the synthesis of non-enzymatic antioxidants (GSH, AA). Moreover, in the sensitive variety, compared to tolerant one, greater changes of the activity of POD and SOD, as well as a higher concentration of H_2O_2 have been shown.

Both varieties differed in the activation of the alternative proline synthesis pathway: in the sensitive variety - an increase, and in the tolerant one - a decrease in the OAT enzyme activity was found. The presence of EBR in the media generally resulted in the reduction of effects caused by ZEA and ROS.

Analyzes of the lipid composition of the tested object membranes showed that under the stress initiated by the ZEA, the percentage share of galactolipids fraction increased, at the cost of the phospholipid fraction in membranes of both varieties, with larger changes in the sensitive variety. The additional presence of EBR in the media reduced the modifications caused by ZEA.

The formation of monolayers from the PL and MGDG fractions, as those occurring in the largest quantities of membranes (Langmuir trough) and measuring for them the isotherms (25°C) - the dependence of surface pressure (π) on the area per single lipid molecule (A),

allowed to determine physicochemical parameters characterizing the structural - mechanical properties of membranes. The most important parameters characterizing the differences in compressibility and flexibility of the membranes were: A_{lim} - the area occupied by the lipid molecule in the maximally "packed" monolayer, as a result of the hydrophilic-hydrophobic interactions between adjacent lipids, π_{col} - pressure corresponding the maximum "packing" of the monolayer and $C_s^{-1}{}_{max}$ - maximum value of the static modulus of monolayer compression.

For both studied lipid fractions, it was shown that the effect of the ZEA was the reduction of the A_{lim} value for both varieties, while changes in the π_{col} parameter differentiated the varieties: a decline of π_{col} was observed for the tolerant and growth – for sensitive variety. The values of $C_s^{-1}{}_{max}$ parameter were dependent on both the type of lipid fraction and the tolerance of objects: for the PL fraction from the tolerant variety a decrease of $C_s^{-1}{}_{max}$ was observed, for the sensitive one - this parameter increased, while changes of the $C_s^{-1}{}_{max}$ were reversed for the MGDG fraction.

Simultaneous exposure of cells to ZEA and EBR resulted generally in smaller (in comparison to ZEA) modifications of physicochemical parameters, especially A_{lim} and π_{col}. ROS application caused, already in the control objects, a reduction in the value of A_{lim} in both varieties, while in the presence of the ZEA and ZEA + EBR, a similar direction of changes of the determined parameters was observed for the not-ozonized objects.

For two-component systems (PL + MGDG), the changes of the excess Gibbs free energy (ΔG^{exc}) were calculated, allowing to determine whether in the mixtures of the composition corresponding to the molar ratio of both fractions determined in biochemical studies, there are attractive interactions between PL and MGDG. In ZEA treated objects, for both varieties a decrease in the ΔG^{exc} value (indicating the attractive interactions) was observed, with a greater decrease was obtained for the tolerant variety, and the presence of EBR reduced the observed effects.

In the ZEA and ROS systems, for the sensitive variety the opposite direction of changes was recorded - the ΔG^{exc} values were positive (indicating the repulsive interactions) while for the tolerant one - no changes were noted (compared to the ZEA system non-treated with ROS).

The obtained results confirm that the presence of ZEA induces oxidative stress generating reactive oxygen species in wheat cells. The differentiation between the tolerant and sensitive varieties, resulting from differences in the composition and structure of membranes, determines a greater accumulation of this mycotoxin in the cells of the sensitive variety, which may be the cause of greater stress effects in this variety. The protection mechanisms include

both enzymatic and non-enzymatic antioxidants, as well as the provision of appropriate osmotic conditions in the cells.

Modifications of cell membranes leading to enlarge the proportion of galactolipid fractions relative to phospholipids, as well as increasing hydrophilic-hydrophobic interactions between lipids reduces the possibility of penetration of this toxin into cells, which can be an important step in the protection mechanism. 24-epibrassinolide applied in hormonal concentrations limits the penetration of ZEA into cells, reducing the stressful effects of this mycotoxin. It can therefore be used as a non-invasive substance in plant protection against ZEA.

On the basis of the obtained data, a scheme of stress and defense mechanisms in a tolerant and sensitive variety after exposure to ZEA stress was proposed.