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The Influence of Selenium on Winter Wheat Resistance to Herbicide Activity in the Forest-Steppe of the Middle Volga Region

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Abstract

One of the major problems that the world is facing today is food security. Despite the fact that herbicides are able to reduce weed plants, they also cause stress to the main crop that the plants' protective system does not always manage to cope with. In this work we present the results of the field experiment on the influence of Na_2SeO_4 (solely and in combination with the herbicide) on winter wheat. Selenium decreases lipid peroxidation by enhancing the activity of the antioxidant enzymes.

Keywords: Enzymes, glutathione reductase, herbicide stress, lipid peroxidation, selenium, TBARS, winter wheat

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Introduction

About half of the agricultural products in the world are produced with the usage of herbicides, because the reduction of weed plants leads to the relatively cheap and effective increase in production (Zhang et al., 2008). However, cultivated crops are experiencing heavy herbicides' pressure (Zarubina et al., 1988).

During the evolutionary process plants have developed a set of non-specific and specific responses, aiming to protect normal state of the intracellular structures and to remove the consequences of the adverse factors. The protective system includes the antioxidant enzymes and low-molecular compounds. However, under the strong influence of the stressor, the protective system does not always handle the load, affecting the formation of plant productivity.

Materials and Methods

The effect of the herbicide on stress-protective mechanisms of winter wheat plants was studied in the field experiment on the black soils of the temperate continental climate area. Spraying was conducted in the tillering stage by Dialen Super (344 grams per liter 2.4 D acid + 120 grams per liter dicamba acid) at a dosage of 0.7 liter per hectare.

The research was conducted on the soils typical for the Middle Volga region: moderately leached chernozem, topsoil consists of humus 5.17 – 6.19%, mobile forms of nitrogen - 106-111 mg/kg, phosphorus 80-93, potassium - 117-140, lead 15.6-17.9 mg/kg, cadmium 0.38 - 0.47 mg/kg, selenium 59-134 mcg/kg of soil; pHкcl 5.0-5.39 (potentiometric), hydrolytic soil acidity 4.95-5.07 mg equivalent, the amount of absorbed bases 27.6-30.70 mg equivalent per 100 g of soil.

One of the indicators that characterize the degree of the damage effect of the unfavorable factors is the intensity of the lipid peroxidation (LPO).

The intensity of this process in wheat leaves was evaluated by the content of thiobarbituric acid-reactive substances (TBARS) - an indirect indicator of changes in LPO.

Lipid peroxidation was estimated by measuring the content of malondialdehyde (MDA) using the thiobarbituric acid (TBA) assays. Winter wheat leaves and root tissues (0.5 g) were extracted in a solution of 3 ml of 1% phosphoric acid, 1 ml of 0.6% (w/v) TBA, 0,1 ml of $FeSO_4 \cdot 7H_2O$ (2.8 mg·ml-1) and 0.3 ml of essence. The mixture was heated for one hour. After cooling it n-Butanol had been added and the mixture was centrifuged for 10 min. The MDA content was estimated by measuring on 532 and 600 nm using «Hitachi-557» spectrophotometer and using a molar absorption coefficient of 155 mM^{-1} cm^{-1}.

Results and Discussion

Analysis of the obtained data (Table 1) showed that plants' spraying with herbicide resulted in the increase of the TBARS content by 34% comparing to the control samples (water spraying).

The content of TBARS in the leaves of the wheat plants sprayed with Na_2SeO_4 (in concentrations 10^{-4} and 10^{-5}%) after 24 hours decreased by 19-22% comparing to the control samples. In plants treated by the mixture of herbicide and Na_2SeO_4 the content of TBARS decreased by 39.6-42.3% comparing to the effect of herbicide alone.

Table 1. *The Content of TBARS, SOD Activity, APX and GR in Winter Wheat Leaves after Herbicide and Na_2SeO_4 Spraying*

Field experiment	Results			
	TBA nmol/g	SOD unit	GR µmol/g	APX µmol/g/min
H_2O-control	392±15	814±45	0.215±0.017	2.64±0.07
Herbicide	525±12	928±36	0.259±0.011	3.16±0.08
$N_2SeO_4 10^{-4}$%	306±19	809±44	0.297±0.019	2.87±0.07
N_2SeO_4 10^{-5}%	318±11	812±21	0.262±0.010	2.73±0.04
$N_2SeO_4 10^{-4}$%+ herbicide	303±9	863±33	0.318±0.009	3.00±0.06
$N_2SeO_4 10^{-5}$% + herbicide	317±14	877±29	0.318±0.016	2.92±0.07

The decrease in concentration of the products of lipid peroxidation in biological membranes under the influence of selenium, in normal conditions as well as under stress, indicates that this trace element either reduces the amount of peroxide oxidation initiators (H_2O_2 , O_2 • -, • OOH, etc), or increases the resistance of biological membranes to oxidative degradation (Seppaenen et al., 2003).

The decrease of the TBRAS production in plants under the selenium influence in stress-free conditions is not related to the deterioration of the metabolic regulations' level in plants, because the growth-promoting and development stimulating solution of Na_2SeO_4 was used in the experiment (same was confirmed by our earlier experiments with other cultures, such as eastern galega, wheat, barley, etc.) (Vikhreva et al., 2002). The 10^{-4} and 10^{-5}% concentrations of Na_2SeO_4 haven't shown signs of plant growth inhibition in normal conditions in any of these cases (Vikhreva et al., 2011).

We can assume, that a decrease of TBARS and O_2^- during the selenate application in the period of stress was based on the same phenomenon - the ability of selenium to immerse plants into a state of deep protective anabiosis during the stress period, characterized by a reduced level of energy production through respiration, and the ability to reduce the superoxide anion production in normal conditions as well as in stress.

The analysis of SOD activity (a single source of superoxide anion production) revealed that SOD activity during the herbicide wheat plants spraying increased by 14%. During the usage of selenium solely the enzyme activity was not changing, and when the combination of herbicide with selenium had been used it increased by 9.0-9.2% in comparison to the control samples.

Our data on the increase of SOD activity in plants under stress complies with the results of other researchers, because the production of the reactive oxygen species can be observed under any stress conditions.

Ascorbate peroxide activity in wheat leaves after the herbicide usage increases by 16%. Plants treatment with selenate, in normal conditions and under stress, caused only a slight increase in the APX activity (by 3 - 7%).

Herbicidal stress caused an increase in the activity of GR by 20.4% compared to the control samples. The impact of selenium on tested plants in stress-free conditions also showed an increase in the activity of GR by 38-22 %, and under stress conditions by 48%, regardless of the concentrations of the Na_2SeO_4 solution used.

This was probably due to the activation of the plant's growth processes by selenium, accompanied, as far as is known, by the increased consumption of reduced glutathione and adequate increase in the formation of oxidized glutathione. Partly, it may be due to the glutathione expenditures on the transformation of selenate ions to selenium containing amino acids.

The increase of glutathione reductase activity in plants fed with selenate under stress corresponds with the increase of the peroxidation processes' level under the herbicidal pressure. It is also possible that plants more actively consume selenate ions under stress, which leads to the accelerated consumption of reduced glutathione.

Reduced glutathione, in addition to participating in the detoxification of the various peroxides and hydroperoxides, extensively expands in the stressful situations, turning into oxidized form (Arora et al., 2002). For this reason, the increase in the activity of the glutathione reductase is likely to be a response to the increase in metabolic activity under the influence of the growth-promoting effect of selenium.

Plants' stress-resistance depends on the ability of plants to survive under the unfavorable conditions and their ability to retain the intensity of destructive processes at a safe for the cell level (Sairam et al., 2000).

The content of TBARS per unit of SOD, GR (in leaves of the control plants and plants treated with Na_2SeO_4) could be an indicator of the wheat plants' adaptation potential, which reflects the level of intensity of lipids peroxidation processes in membranes (Table 2).

Table 2. *The Content of TBA per Unit of SOD, GR, APX*

Field experiment	TRARS, nmol/g		
	SOD, unit	GR, µmol/g/min ($1\ 10^{-2}$)	APX, µmol/g/min ($1\ 10^{-1}$)
H_2O-control	0.48	18.23	14.85
Herbicide	0.62	22.20	18.20
$N_2SeO_4 10^{-4}$%	0.38	10.33	10.66
$N_2SeO_4 10^{-5}$%	0.39	12.10	11.65
$N_2SeO_4 10^{-4}$% + herbicide	0.35	9.50	10.10
$N_2SeO_4 10^{-5}$% + herbicide	0.36	10.0	10.90

Under the influence of Na_2SeO_4, the intensity of peroxide processes, with respect to the GR enzymes' activity, decreased. In our opinion, this may be a reflection of the ability of selenium to change the redox status of the cell, and to control the level and concentration of reactive oxygen species, influencing the value of the ratio of reduced and oxidized (G - SH / G - SS - H) glutathione.

Herbicides' stress effect, even though it is beneficial to weed reduction, may result in the decline of the main crop yield by 35-50%, so recent trends are use herbicides in combination with the anti-stress agents, in case of Na_2SeO_4 the effective concentration is 10^{-4}-10^{-5}% (or 200 ml per ha).

Conclusions

Plant resistance to stress caused by herbicides was due to the plant antioxidant enzyme system's ability to maintain destructive processes on the safe level for the cell. Antioxidant enzymes activity in leaves, as well as in roots of the seedlings on the initial stage of ontogenesis can be used as an indirect mark of the lipid peroxidation caused by the herbicides' application.

Joint application of selenium and herbicides causes a reduction of lipid peroxidation products in wheat leaves. The decrease of the TBARS level was determined by the dose of selenium. The application of selenium in low doses (10^{-4}%) on soils polluted with herbicides may decrease or restrain lipid peroxidation induced by pollution, higher doses of selenium (10^{-2}%) weakened adaptive capacity of winter wheat plants on early stages of ontogenesis.

The influence of selenium on exogenous enzymes activity for detoxification of reactive oxygen species indicates the ability of this element to adjust the redox status of the cells towards the increase of stress tolerance.

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