UDC: 581.1:633.16:632.954:631.811.98

THE ACTIVITY OF SEPARATE ANTIOXIDANT ENZYMES OF OXIDOREDUCTASES CLASS USING HERBICIDE KALIBR 75 AND PLANT GROWTH REGULATOR BIOLAN

V.P. KARPENKO, Doctor of Agricultural Sciences
R.M. PRYTULYAK, A.O. CHERNEHA, Candidates of Agricultural Sciences

The results of studies on the effects of various norms of herbicide Kalibr 75 (40, 50 and 60 g/ha) and methods of application of plant growth regulators Biolan (spraying before sowing seeds – 20 ml/t spraying – 10 ml/m) on the activity of antioxidant enzymes of oxidoreductases class (catalase, peroxidase) are presented.
Keywords: *winter barley, herbicide, plant growth regulator, enzymes.*

It is known that the period of use of chemical protection of crops from weeds is more than fifty years, as a result of which were occurred and continue to occur significant changes not only in the weeds that produce a protective reaction to the action of herbicides and become resistant to a number of chemical classes preparations, but also in crop plants. However, in contradistinction to weed, crops during ontogeny can't quickly develop protective mechanisms on the action of herbicides, which are new factor for them. Therefore, the herbicides used in growing every single culture, is a xenobiotic for it and during the wrong application are capable to predetermine stress. Crop plants adapt to the effect of xenobiotics, primarily due to numerous adaptive mechanisms that have emerged during their evolution. The more adaptation mechanisms are used by a plant simultaneously at different levels, the more resistant an organism becomes to separate factor, and their complex [1].

Biodegradation of xenobiotics in plant cell occurs in the endoplasmic reticulum, resulting in possible increased generation of superoxide anion O^{-2}. This radical has weak redox properties and rarely causes damage to biological molecules. However, O^{-2} serves as a source for the formation of hydrogen peroxide in the cell, which is a highly toxic compound.

Plant organisms have sufficient resistance to oxidative damage due to the presence in the cell of antioxidant systems composed of individual antioxidant enzymes of oxidoreductases class. Among them, an important role in the detoxification of hydrogen peroxide plays catalase, which converts hydrogen peroxide into water and oxygen, and the various peroxidase which are present in many compartments of plant cell and recover H2O2 to H2O [2].

It is known that xenobiotics, which also include herbicides, can influence the course of physiological and biochemical processes in crops. One of the negative effects of the herbicide is development of stress in crops, during which in photosynthetic tissues, concentration of reactive oxygen species (ROS) increased [3]. ROS are capable of reacting with proteins, lipids, nucleic acids, resulting in a

number of destructive processes in the plants: chlorophyll photooxidation, lipid peroxidation and sulfhydryl groups of proteins of chloroplast membranes, breach of chloroplast DNA structure and so on. [4, 5].

The level of ROS in cell is controlled by antioxidant systems that include low molecular antioxidants and antioxidant enzymes [6, 7]. In particular, in the inactivation of ROS involved catalase enzymes, peroxidase and ascorbate oxidase [8, 9] and low molecular antioxidants – ascorbate and glutathione [10 – 13].

The reaction of antioxidant enzyme systems of the different types of herbicides was investigated in many crops: spring barley, wheat and winter triticale and others [14, 15]. However, with the mass use of herbicides, the study of measures that enhance physiological and biochemical status of plants and are able to influence their protective and adaptive response to xenobiotics is of particular interest. One such measure is the use of plant growth regulators with herbicides. This increases the stability of crops to stress factors, which apparently is provided by the growth under the influence of PGR activity of redox enzymes.

It is proved that tank mixtures of herbicides and PGR positively affect the enzyme activity in crops [16]. However, in the works of V. Ladonin and others [17] observed that in sensitive pea plants under the influence of 2,4-D peroxidase activity is changed, but in barley plants herbicide didn't affect the distribution of peroxidase and catalase activity.

Based on the above mentioned material, an objective of our researches was to determine how different norms of herbicide Kalibr 75 and methods of use of PGR Biolan affect the activity of some antioxidant enzymes of oxidoreductase class (catalase, peroxidase, ascorbate oxidase) in winter barley plants grown in vegetation conditions.

Research Methods. Investigation of the herbicide and PGR activity performed using winter barley plants (*Hordeum sativum* Jessen) of variety Dostoynyy that was grown in plastic containers with podzolized heavy loamy soil with the compliance of requirements of the growing method [18]. The scheme of the experiment consisted of 16 variants presented in the tables. Herbicide Kalibr 75 in norms 40; 50 and 60 g/ha was applied both separately and in mixtures with PGR Biolan in the phase of plants tillering; in the fifth variant Biolan was used separately – by spraying plants; in 9 – 16 variants – used seed application with Biolan before sowing at the rate of 20 ml/m (background); in variants in 10 – 12 applied herbicide Kalibr 75 in norms 40; 50 and 60 g/ha on the background; in 13th variant applied on the background only Biolan and in variants 14 – 16 – Kalibr 75 40; 50 and 60 g/ha was applied with Biolan in norms 10 ml/ha on the background.

Activity of oxidoreductase class enzymes – catalases (EC 1.11.1.6), peroxidases (EC 1.11.1.7) in leaves of winter barley was determined in leaves samples selected on the third and tenth day after applying preparations by the methods described by Kh.M. Pochynok [19].

Research Results. The study of separate enzymes in the oxidoreductase class in winter barley plants made in strictly controlled conditions, showed the dependence of their activity from the herbicide norms applied separately and in mixtures with PGR Biolan. Thus, the analysis of obtained data of vegetation

experiment in 2010 showed that on the third day after application of the herbicide Kalibr 75 in norms 40; 50 and 60 g/ha, catalase activity increased with the increase of preparations norms 20.9; 36.9 and 52.5 micromoles of decomposed H_2O_2 respectively comparing to the control (Table 1).

1. Catalase activity in leaves of winter barley during the activity of herbicide Kalibr 75 and PGR Biolan, micromoles of decomposed H_2O_2/g of raw mass for 1 min.

Research variant	2010		2011		2012	
	On the third day	On the tenth day	On the third day	On the tenth day	On the third day	On the tenth day
Processing with water (control)	84,7	115,4	45,3	123,7	52,1	74,5
Kalibr 75 40 g/ha	105,6	136,1	65,4	145,2	74,1	96,5
Kalibr 75 50 g/ha	121,6	142,3	78,1	162,3	86,2	123,1
Kalibr 75 60 g/ha	137,2	156,1	84,1	175,3	95,3	132,8
Biolan 10 ml/ha	110,4	126,7	52,4	136,7	64,7	81,6
Kalibr 75 40 g/ha + Biolan	121,4	142,1	75,1	159,6	89,7	112,4
Kalibr 75 50 g/ha + Biolan	145,7	154,1	88,9	178,1	98,1	132,7
Kalibr 75 60 g/ha + Biolan	157,1	164,9	95,4	179,2	112,4	136,2
Biolan 20 ml/t — seed treatment (background)	118,3	130,4	56,7	139,7	68,9	86,7
Background + Kalibr 75 40 g/ha	132,4	145,7	79,1	168,7	91,2	123,4
Background + Kalibr 75 50 g/ha	150,3	162,1	96,4	184,2	113,4	149,3
Background + Kalibr 75 60 g/ha	167,2	170,8	104,2	195,1	123,7	156,7
Background + Biolan 10 ml/ha	129,4	135,2	63,4	145,7	74,2	96,3
Background + Kalibr p 75 40 g/ha + Biolan	145,2	152,3	89,7	184,1	96,5	142,3
Background + Kalibr p 75 50 g/ha + Biolan	162,1	172,6	112,4	196,2	123,1	156,7
Background + Kalibr 75 60 g/ha + Biolan	178,4	186,6	123,6	201,5	132,5	185,9
NIR_{01}	*4,2*	*5,1*	*2,4*	*3,7*	*2,3*	*2,9*

Using herbicide KAlibr 75 in norms 40; 50 and 60 g/ha in mixtures with Biolan 10 ml/ha, catalase activity increased relative to variants with the application of herbicide only on 15.8; 24.1 and 19.9 micromoles decomposed H_2O_2 respectively. During application of the same norms of the herbicide, but on the background of seed treatment before sowing PGR Biolan in the norm 20 ml/t, catalase activity compared with the control increased by 47.7; 65.6 and 82.5 micromoles decomposed H_2O_2 respectively.

The highest catalase activity in leaves of winter barley was noted after the use of the herbicide Kalibr 75 in norms 40; 50 and 60 g/ha of seed treatment on the background of PGR Biolan and its application on sprouts. With the integrated use of preparations, catalase activity in comparison with control increased to 71; 91 and 111%.

On the tenth day after application of the herbicide and the PGR, level of catalase activity in leaves of winter barley compared to the third day rose, but revealed regularity of action of various norms of Kalibr 75 applied separately and in combination with Biolan is preserved. Thus, according to the norms of Kalibr 75 40; 50 and 60 g/ha applied without Biolan, catalase activity was 136.1; 142.3 and 156.1 micromoles of decomposed H_2O_2 /g of raw mass per min., and after the

combined use of the same herbicide with PGR on the background of applying seeds with Biolan – 152.3; 172.6 and 186.6 micromoles 115.4 micromoles of decomposed H_2O_2 in control and NIR_{01} = 5.1.

1. Peroxidase activity in leaves of winter barley during the activity of herbicide Kalibr 75 and PGR Biolan, micromoles of oxidized guaiacol /g of raw mass for 1 min.

Research variant	2010		2011		2012	
	On the third day	On the tenth day	On the third day	On the tenth day	On the third day	On the tenth day
Processing *with water (*control)	75,2	97,1	62,3	77,4	73,2	103,1
Kalibr 75 40 g/ha	96,3	123,4	89,4	89,1	115,3	137,6
Kalibr 75 50 g/ha	112,8	137,9	96,5	112,3	119,1	154,8
Kalibr 75 60 g/ha	126,3	145,2	103,7	118,7	129,4	159,1
Biolan 10 ml/ha	86,4	112,3	71,6	85,1	88,6	124,8
Kalibr 75 40 g/ha + Biolan	123,4	132,2	98,7	96,3	121,9	146,3
Kalibr 75 50 g/ha + Biolan	138,1	148,7	115,9	117,3	145,7	165,3
Kalibr 75 60 g/ha + Biolan	147,6	154,4	126,8	126,9	152,4	171,6
Biolan 20 ml/t — seed treatment (background)	89,3	116,6	78,0	87,1	96,3	129,6
Background + Kalibr 75 40 g/ha	126,5	133,4	106,3	102,3	131,2	152,3
Background + Kalibr 75 50 g/ha	140,1	152,1	118,2	116,3	147,2	169,5
Background + Kalibr 75 60 g/ha	152,3	158,7	135,8	131,7	167,3	176,4
Background + Biolan 10 ml/ha	96,1	119,2	79,2	92,3	97,8	132,9
Background + Kalibr p 75 40 g/ha + Biolan	132,2	145,3	112,3	109,6	138,9	161,5
Background + Kalibr p 75 50 g/ha + Biolan	153,4	164,8	124,6	121,4	151,4	183,4
Background + Kalibr 75 60 g/ha + Biolan	168,9	174,9	143,1	138,4	178,8	190,2
NIR_{01}	*13,2*	*9,8*	*11,7*	*11,2*	*12,3*	*6,8*

This dependence of catalase activity was observed in 2011 and 2012, but the highest it was in variants where Kalibr 75 in norms 40; 50 and 60 g/ha was applied

together with Biolan on the sprouts on background of applying seeds with PGR. From the obtained data we can see that the combination of herbicide application and PGR on the background of applying seeds with PGR provides a significant increase in catalase activity in leaves of winter barley, which can indicate rise in level of detoxification processes in plants, aimed at elimination of the negative impact of one of the products of metabolism of herbicides – H_2O_2. Obviously, against the background of applying seed with PGR, in plants creates greater energy potential, focus of which after the use of herbicide and PGR during the growing season of barley, provides more distinct rates of detoxification of xenobiotics.

Peroxidase activity (Table 2) in the studied variants also exceeded control indices on the third, and on the tenth day after application of the herbicide. In particular, in 2010, after applying Kalibr 75 in the norms of 40 to 60 g/ha on the third day of peroxidase activity increased respectively from 96.3 to 126.3 micromoles, and after combined usage of Kalibr 75 in the investigated norms in tank mixtures with Biolan, peroxidase activity increased from 123.4 to 147.6 micromoles of oxidized guaiacol respectively.

Application of herbicide Kalibr 75 in norms 40; 50 and 60 g/ha on the background of seed treatment with PGR Biolan increases activity of peroxidase in comparison with the control to 51.3; 64.9 and 77.1 micromoles of oxidized guaiacol respectively.

Significant increase in enzyme activity was observed after the combined application of Kalibr 75 with Biolan during the vegetation period of the crop on the background of PGR seed treatment before sowing. Thus, mixtures of herbicide Kalibr 75 with Biolan predetermined increase of peroxidase activity compared to the control within 76 to 125%.

On the 10^{th} day of accounting, peroxidase activity significantly exceeded the indices of the control variant, but the highest it was after the combined use of Kalibr 75 (40, 50 and 60 g/ha) with PGR Biolan added on the background of applying seeds with plant growth regulator. Thus, at this combination of preparations, the excess before the control was 50 – 80%. Such dependences of peroxidase were manifestated during the action of preparations in 2011 and 2012.

Conclusions. Herbicide Kalibr 75 in norms 40, 50 and 60 g/ha has a positive effect on the metabolic reactions manifested in the activation of separate enzyme of oxidoreductase class (catalase, peroxidase) and can testify about increase of level of detoxification processes in the plant organism. The combination of the application of different norms of herbicide Kalibr 75 with PGR Biolan causes increase of activity in winter barley plants of enzymes catalase and peroxidase, but their activity depends on the norm of use of Kalibr 75 and combination of these norms of preparation usage with various ways of applying PGR Biolan: with the increase of norms of applying Kalibr 75 to 60 g/ha enzyme activity in plants increases significantly. Increase of enzyme activity is observed also during the joint use of Kalibr 75 with PGR Biolan, particularly on the background of applying Biolan on seeds, which indicates that the increase of the antioxidant status of plants with the active participation of these enzymes in plant adaptation to herbicide stress.

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