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The increase of phytotoxic action of graminicide fenoxaprop-P-ethyl by NO donor sodium nitrpruside

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Abstract. It was established, that pretreatment of oat plants by NO donor sodium nitroprusside (SN) increased their sensitivity to the herbicide fenoxaprop-P-ethyl (FP) action. Received data proved the possibility of increase of herbicide inhibitors of acetyl-coA-carboxylase phytotoxic action due to the impact on NO-signalling system.

***Keywords**: NO, sodium nitroprusside, programmed cell death, graminicides, induced pathogenesis*

Introduction. Herbicides site of action of which is enzyme acetyl-coA-carboxylase (ACC) are the most effective modern herbicides. Inhibitors of ACC have been combined in the group of graminicides because due to site of action peculiarities, only plants of cereal family are sensible to them [10]. The decrease of the weed control efficiency under the stress conditions and antagonistic interaction with herbicides effective against dicot weeds are essential disadvantages of graminicides [2]. It was established that antagonistic decrease of herbicides phytotoxic action is not related to the decrease of their inhibitory effect to ACC activity [8], so it must be determined by changes in induced pathogenesis prosess. Therefore when using graminicides for the development of effectiveness prevention methods, it was necessary to uncover the nature of pathogenesis induced by those herbicides.

It was established that development of graminicide's phytotoxic action is mediated by active oxygen forms (AOF) formation [3, 4]. Also it has been shown that herbicide inhibitor of acetyl-coA-carboxylase haloxyfop-R-methyl have been causing internucleosome DNA fragmentation of maize seedlings root merystem [6]. Obtained data has shown that AOF-mediated programmed cell death (PCD) process take part in graminicides induced pathogenesis. It followed that increase of the graminicides phytotoxic action can be achieved by shift of anti-prooxidant balance to the prooxidant processes direction. This hypothesis has been confirmed by the research of graminicides phytotoxic action changes in mixtures with herbicides with prooxidant activity [5]. However, the possibilities of such a way of graminicides phytotoxic action increase are limited by prooxidant herbicides selectivity to the certain species of crops. In this connection, the possibility of increase of graminicides phytotoxic action through the application of nonphytotoxic compound with prooxidant activity is relevant. In the conditions of laboratory experiments, the nonphytotoxic concentration of hydrogen peroxide significantly accelerated the appearance of maize seedlings root apical merystem necrosis [3]. However, the plants treatment with hydrogen peroxide haven't been resulted in essential increase of graminicides phytotoxicity in the vegetative investigation and in the field. Probably it is related with rapid exogenous hydrogen peroxide decomposition by antioxidant enzymes and substantial activity of anti-prooxidant balance support system state (data not published). The influence on NO-synthase signalling sysytem may be an alternative way of prooxidant application for stimulation of graminicides-induced pathogenesis, since it is known, that this system take part in plant programmed cell death initiation [1, 9; 12].

Research purpose. In this regard the aim of our study was to investigate the effect of NO donor sodium nitroprusside (SN) ($(Na_2\ Fe(NO)(CN)_5$) on graminicide fenoxaprop-P-ethyl (FP) phytotoxic action.

Materials and methods. As the object of research, as annual cereal weeds model, oat plants (*Avena sativa* L.) sensitive to FP had been used. Plants were grown in vegetative area of Institute of plant physiology and genetics in plastic pots with capacity 1 L using a mixture of soil and sand in the ratio of 1:3 under natural light. The treatment was carried out in three leaves phase by spraying of SN solution in 2 mM concentration and FP in $5\cdot10^{-5}$ and 10^{-4} M concentration. The total volume of solution for spraying was 9 ml. The treatment of NS solution was performed 24 hours prior to FP treatment. The inactivated SN solution kept in light during the day was used to confirm the induction of herbicide's phytotoxicity changes by NO molecule [11].

The phytotoxic action was determined on 21-st day after treatment by the growth inhibition of the aboveground part wet substance weight and by decrease of photosynthetic pigments content in plant leaves. The photosynthetic pigments content was determined by plant material extraction in DMSO [13]. The investigations were carried out independently 4 times, the statistical data processing was performed by Exel.

Table 1.

Weight of the oats plant aboveground part after herbicide FP, SN and inactivated SN action (g).

№	Variant experiment	Weight of one plant, g	Phytotoxicity, % to control
1	control	0,243	100
2	FP ($5\cdot10^{-5}$ M)	0,199	18
3	FP (10^{-4} M)	0,055	77
4	FP ($5\cdot10^{-5}$ M)+SN (2mM)	0,119	51
5	FP (10^{-4} M)+ SN (2mM)	0,046	81
6	FP ($5\cdot10^{-5}$ M)+ SN (2mM) (inactivated)	0,176	28
7	FP (10^{-4} M)+ SN (2mM) (inactivated)	0,041	83

Results and discussion. The treatment with SN in 2 mM concentration had no effect on oats plant weight. FP in 10^{-4} M concentration caused the total plant death. Total leaves necrosis had been observed, and wet substance weight was 77 % lower that control (Table 1). The treatment with SN had no effect on phytotoxic action at this FP concentration. The decreasing of FP concentration to $5\cdot10^{-5}$ M led to respective phytotoxic action decrease: wet

substance weight was 18 % of control, the edges of leaves were partially necrotic on 21-st day after treatment. Stimulating effect of SN pretreatment on phytotoxic action progress with a reduced FP concentration had been distinct. The phytotoxic action increased by 32 % compared with the effect of FP that determined by the degree of plant weight growth inhibition. Inactivated SN did not affect the plant weight growth inhibition by FP significantly. Similar results were obtained in determination of FP phytotoxicity by the photosynthetic pigments content reduction. The content of chlorophyll a under SN pretreatment was 1,5 times less than under herbicide treatment in $5 \cdot 10^{-5}$ M concentration, while in inactivated SN variant chlorophyll content was not significantly different from FP variant (Table 2).

Conclusions. FP phytotoxic action increase on account of NO donor SN pretreatment on condition that SN inactivation causes to loss of this effect, suggests that herbicides inhibitors of acetyl-coA-carboxylase phytotoxicity may be increased by influencing NO-synthase signaling system. It can be assumed that stimulation of graminicides phytotoxic action by NO donor is associated with induction of apoptosis, based on the initiation of PCD by NO-synthase signaling system data [1, 9, 12]. However, it is known that NO can also affect the state of the antiprooxidant balance, thus depending on the concentration it can act both as antioxidants and as a pro-oxidant [7]. Consequently, the influence of NO donors on the graminicides phytotoxic effect may be mediated by the interaction of NO-synthase signalling system with ROS. Therefore, for clarification of the mechanism of NO donors influence on the phytotoxic action of herbicides inhibitors of acetyl-CoA carboxylase it is necessary to conduct further investigations.

Table 2.

The pigments content (mkg/mlg of wet substance) in the oat leaves after FP and SN treatment on 21-st day.

№	Variant	Chlorophyll a	Chlorophyll b	Total chlorophyll content (a+b)
1	control	8,40	1,95	10,35
2	FP ($5 \cdot 10^{-5}$ M)	3,47	0,42	3,89
3	FP (10^{-4} M)	0,66	0,41	1,07
4	FP ($5 \cdot 10^{-5}$ M)+SN (2mM)	2,12	0,70	2,82
5	FP (10^{-4} M)+ SN (2mM)	0,34	0,37	0,72
6	FP ($5 \cdot 10^{-5}$ M)+ SN (2mM) (inactivated)	2,87	1,17	4,04
7	FP (10^{-4} M)+ SN (2mM) (inactivated)	0,35	0,34	0,69

REFERENCES (TRANSLATED AND TRANSLITERATED)

1. Dubovskaya L.V., Kolesneva E.V., Knyazev D.M., Volotovskyi I.D. Zashchitnaya rolj oksida azota pri okisliteljnom stresse, indutsyrovanom v rasteniyah tabaka peroksidom vodoroda [The protective role of nitrogen oxide under condition of oxidative stress induced by hydrogen peroxide in tobacco plants] // Fisiologiya rastenyi. – 2007. – T. 54. – S. 847–855.
2. Morderer Ye.Yu. Merezhynskiyi Yu. G. Gerbitsydy. T.1. Mehanismy diyi ta praktyka zastosuvannya [The mechanisms of action and applying practice]. K.: Logos, 2009. – 379 s.
3. Morderer Ye.Yu., Palanytsya M. P., Rodzevich Ye. P. Doslidzhennya uchasti viljnoradykaljnyh okysnyuvaljnyh reaktsiyi u rozvytku fitotoksychnoyi diyi graminitsydiv [The investigation of free radical oxidative reaction participation in graminicides phytotoxic action progress] // Fisiologiya i biohimiya kuljturnyh rasteniyi. – 2007. – T. 54. S. 847–855.
4. Palanytsya M. P., Trach V. V., Morderer Ye.Yu. Generuvannya aktyvnyh form kysnyu za diyi graminitsydiv i modyfikatoriv yih aktyvnosti [The generation of active oxygen forms after graminicides and their modifiers action] // Fisiologiya i biohimiya kuljturnyh rasteniyi. – 2009. – 41, № 4 – S. 328-334.
5. Radchenko M. P., Sychuk A. M., Rodzevich Ye. P., Morderer Ye.Yu. Pidvyshchennya vybirnoyi fitotoksychnosti ta stan prooksydantno-antyoksydantnoyi rivnovagy v razi zastosuvannya graminitsydu fenoksaprop-P-etylu v potriyiniyi sumishi z gerbitsydamy synergistom ta antagonistom [Increased selective phytotoxicity and the prooxidant-antioxidant balance in the case of graminicide fenoxaprop-P-ethyl applying in triple mixture with sinergist and antagonist] // Fisiologiya i biohimiya kuljturnyh rasteniyi. – 2013. – 45, № 4 – S. 306 – 312.
6. Sychuk A. M., Radchenko M. P., Morderer Ye.Yu. Zaprogramovana zagybelj klityn u patogenezi, indukovanomu gerbitsydamy – ingibitoramy acetyl-koA- karboksylazy [The programmed cell death in the pathogenesis induced by herbicides – inhibitors of acetyl-CoA carboxylase] // Biologichni studiyi. – 2013. – 2. – S. 101-106.
7. Beligni M., Lamattina L. Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues // Planta. –1999. – Vol. 208. – P. 337–344.
8. Bjelk L., Monaco T. Effect of chlorimuron and quizalofop on fatty acid biosynthesis // Weed Sci. – 1992. – V. 40. – P. 1–6.
9. Clarke A., Desikan R., Hurst R., Hancock J., Neil S. NO way back: nitric oxide and programmed cell death in Arabidopsis thaliana suspension cultures // Plant J. – 2000. – Vol. 24. – P. 667–677.
10. Delye C. Weed resistanse to acetyl coenzyme A carboxylase inhibitors: un update // Weed Sci. – 2005. – Vol. 53. – P. 728–746
11. Friederich G., Butterworth J. Sodium nitroprusside: twenty years and counting // Anesth. Analg.– 1995.– V.81. – № 1.– P. 152–162.
12. Pedroso M., Magalhaes J., Durzan D. Nitric oxide induces cell death in Taxus cells // Plant Sci. – 2000. – Vol. 157. – P.173-180.
13. Welburn A.R. The spectral determination of chlorophylls a and b as well as total carotenoids with spectrophotometry of different resolution // J. of Plant Physiol. – 1994. – Vol. 144. – № 3. – P. 248-254.

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Повышение фитотоксического действия граминицида феноксапроп-Р-этила донором NO нитропруссидом натрия

Аннотация.Установлено, что предварительная обработка растений овса донором NO нитропруссидом натрия (НПН) повышает их чувствительность к действию гербицида феноксапроп-Р-этила (ФП). Полученные данные подтверждают возможность повышения фитотоксического действия гербицидов ингибиторов ацетил-КоА-карбоксилазы за счет влияния на NO-сигнальную систему.

Ключевые слова: *NO, нитропруссид натрия, программированная гибель клетки, граминициды, индуцированный патогенез.*