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DNA FRAGMENTATION AND ENDONUCLEASE ACTIVITY UNDER THE EFFECT OF HERBICIDES ACETYL-CoA-CARBOXYLASE AND ACETOLACTAT SYNTHASE INHIBITORS

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The aim of this study was to test the hypothesis that pathogenesis induced in sensitive plants by ALS- and ACC-inhibiting herbicides is an active process which involves programmed cell death (PCD). A reliable marker of PCD is DNA fragmentation, which is carried out by endogenous nucleases. The root meristems from herbicide-sensitive plants were analyzed by the TUNEL assay to estimate DNA fragmentation. It was shown that the highest level of DNA fragmentation in root meristem cells under pathogenesis, induced on maize seedlings by the action of ACC-inhibiting herbicide propaquizafop, and on pea seedlings by the action of ALS-inhibiting herbicide tribenuron-methyl, was associated with increased total nuclease activity in root meristem lysates comparing to the corresponding control. In response to tribenuron-methyl application, the level of total nuclease activity elevated due to high activity of both Zn^{2+}-dependent («acidic») and Ca^{2+}/Mg^{2+}-dependent («alkaline») nucleases, while in a case of propaquizafop, the increased activity was detected only for Zn^{2+}-dependent nucleases. These differences may be due to different mechanisms of PCD initiation, depending on the mode of herbicides action. The facts, that TUNEL method recorded DNA fragmentation in plant cells induced by ACC- and ALS-inhibiting herbicides, and this fragmentation is associated with an increased activity of endogenous nucleases, represent evidence for PCD, that occurs during pathogenesis. The significance of data obtained is that they emphasize the fact that herbicide-induced pathogenesis is a complex, multi-stage, active process. The discovery of pathogenesis distinct stages mechanisms nature opens up new possibilities for regulating the herbicides selective phytotoxicity by physiologically active substances and genetically engineered manipulations.

Key words: DNA fragmentation, endonucleases, programmed cell death, herbicides, propaquizafop, tribenuron-methyl.

Traditionally the research in physiology of herbicides action focuses on the identification of herbicides sites of action, and on the processes that determine the herbicides concentration at these sites. However, it has been shown that the reduction of phytotoxic action of acetyl-CoA-carboxylase

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(ACCase) inhibitors on grass weeds under drought conditions, or in tank mixtures with herbicides effective against broadleaf weeds, is not associated with significant changes in their uptake, detoxification, or decrease of ACCase activity inhibition [1]. Thus, the resulting phytotoxicity of ACCase-inhibiting herbicides is not solely determined by the inhibitory activity at the site of action, but depends on the development of the pathogenesis induced by them [2]. In this regard, the mechanisms of pathogenesis, induced by herbicides with different modes of action, are of clear interest.

Currently, available information about the mechanisms of herbicide-induced pathogenesis is scarce. In particular, the involvement of programmed cell death (PCD) in the pathogenesis induced by some herbicides has been established. It has been found, that upon introduction of the animal anti-apoptotic gene *BC1-2* into tobacco chloroplasts, their resistance to such herbicides as paraquat (electron interceptor from the primary acceptor of photosystem I), acifluorfen, sulfentrazone (protoporphyrinogen oxidase (PPO) inhibitors) increased. While tobacco wild-type plants died under the action of these herbicides exhibiting features associated with apoptosis, transgenic plants survived without manifestation of any apoptotic-like characteristics [3]. After treatment of tobacco plants with herbicide paraquat they demonstrate the release of phytaspase (cell death promoting plant protease with caspase specificity) from the apoplast into the cells cytoplasm [4]. After the introduction of anti-apoptotic *p35* baculovirus gene in passion fruit the plant resistance to herbicide glufosinate increased [5]. These data provide clear evidence supporting the involvement of apoptotic-like PCD in the induced pathogenesis, but they include only herbicides, which phytotoxic effect occurs due to the photosynthesis disorganization and the direct formation of reactive oxygen species (ROS) [6].

The involvement of PCD in the pathogenesis induced by other classes of herbicides, including the herbicides of the two most effective and widely used classes — acetyl-CoA-carboxylase (ACC) and acetolactate synthase (ALS) inhibitors, remained questionable. It was shown essential transcriptional changes related to ribosome biogenesis and translation, secondary metabolism (detoxification of herbicides), cell wall modification, and growth in response to herbicides ALS inhibitors in plants [7], however there are no data on the expression of genes responsible for PCD. One of the reliable markers of apoptotic-like PCD is the DNA degradation into oligonucleosomal units («DNA ladder») and activation of endogenous nucleases, involved in this degradation. For the ACCase-inhibiting herbicides such DNA degradation was detected using TUNEL assay when their phytotoxic action was enhanced synergistically in the mixtures with dinitroaniline-derived herbicides [8].

To determine the involvement of PCD in the pathogenesis induced by herbicides ACC and ALS inhibitors an electrophoretic study of DNA degradation was performed. The effect of ACCase-inhibiting herbicide halaxyfop-methyl was investigated on the meristem of maize seedlings, and ALS-inhibiting herbicide tribenuron-methyl action was studied on sunflower leaves [1, 9]. It was found, that for 2 days after treatment by halaxyfop-methyl, DNA extracted from the meristem of maize seedlings remained intact. On the 3rd day, partial degradation of DNA was observed,

but without fragmentation into oligonucleosomal units. Starting from the 4th day DNA fragments, which sizes corresponded approximately to 180—200 bp (the size of nucleosome), were detected on electrophoregram [1]. Under tribenuron-methyl treatment DNA degradation in the cells of sunflower leaves occurred in a similar way, but developed slightly slower: internucleosomal fragmentation was observed on the 6th day after plants treatment by herbicide. The obtained data suggests that initially the cell death under the effect of herbicides ACC and ALS inhibitors occurs by the mechanism of necrosis, which is accompanied by DNA degradation without internucleosomal DNA fragmentation. However, apoptotic-like PCD may also have some involvement in the process of pathogenesis as evidenced by internucleosomal DNA fragmentation at the late stages of phytotoxic action [9].

Cell destruction can manifest as apoptotic-like, necrotic or autophagic cell death, and these processes are likely to overlap extensively, sharing several regulatory mechanisms [10]. But until recently, the term PCD was used to describe the processes of apoptosis and autophagy, while necrosis was described as a chaotic and uncontrolled mode of death [11]. Apoptosis was considered as energy-dependent process that principally distinguishes it from necrosis, which is characterized by common deficiency of intracellular energy due to blocking of mitochondrial respiration and/or glycolytic ATP generation [12]. On the other hand, there is an opinion that the differences between necrosis and apoptosis are not qualitative but rather quantitative, so therefore necrosis can be assumed as an actively regulated process and qualified as a separate form of PCD [13]. However, this concept requires further investigation in plant systems.

The aim of this study was to investigate whether the plant DNA degradation induced by the ACC- and ALS-inhibiting herbicides is a result of endogenous nuclease activation. Although necrosis is considered now as one of the types of PCD, the fact that DNA fragmentation is mediated by the activation of endogenous nucleases may provide additional evidence that pathogenesis, induced in sensitive plants by herbicides ACC and ALS inhibitors, is an active regulated process that proceeds through the mechanism of PCD.

Materials and Methods

Herbicide treatment. Root meristems of herbicide-sensitive plant species were used. The propaquizafop (Agil 100 EC; 100 g/l a.i., EC; ADAMA) effect on DNA was assessed using maize seedlings (*Zea mays* L., variety Igumets) as a monocot model. Pea seedlings (*Pisum sativum* L., variety Gotovsky) as a dicot plant model were used for tribenuron-methyl (Granstar 75 %; 750 g/kg a.i., WG; DuPont) herbicide action assessment. The seeds for treated seedlings groups were soaked in solutions of corresponding herbicides ($5 \cdot 10^{-5}$ M), and for control group the seeds were soaked in water for 20 h. Herbicide concentrations were selected based on our previous investigations [14]. Afterwards, the seeds were washed and germinated in four replications for each variant in thermostat at 24 °C for maize seedlings and at 18 °C for pea seedlings. Samples for analyzes were taken on the 3rd, 4th, 5th day after treatment.

DNA fragmentation analysis. The involvement of PCD in herbicide-induced phytotoxicity was evaluated *in situ* by the level of DNA fragmentation in the plant cells using the TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling) assay. TUNEL assay is based on the incorporation of labeled dUTP (deoxyuridine triphosphate) in DNA (mediated by the enzyme terminal deoxynucleotidyl transferase), which occurs only at the regions with free 3′-hydroxyl terminations (i.e., breaks or extreme ends of chromosomal DNA) [15]. TUNEL analysis was performed by the assay of Tripathi [16], which has been optimized for plant objects, containing solid cellulosic cell walls.

Fixation. The roots of the plants treated with the appropriate herbicides were fixed in fixating solution (4 % paraformaldehyde in 1x PBS (phosphate buffered saline, pH = 7.4)) by incubating for 16 h at 4 °C. Fixation was performed in 50 ml test-tubes with their caps closed properly. Fixed roots were washed with 96 % ethanol (roots were gently shaked for 10—15 seconds in ethanol) twice at room temperature. After washing roots samples were stored in 70 % ethanol for 24 h at 4 °C. Ethanol solution was decanted and roots were incubated in 1x PBS for 20 min at room temperature. PBS was removed and this step was repeated with fresh PBS to completely remove traces of ethanol.

Permeabilization. In order to permeabilize plant cell wall, roots were incubated overnight at 37 °C in 5 mg/ml glucanase solution in 100 mM sodium citrate buffer (pH = 6.0). Solution was removed and roots were incubated in permeabilization solution (0.1 % Triton X-100 in 100 mM sodium citrate buffer, pH = 6.0) at 37 °C for 30 min in water bath with intermittent shaking. To digest the proteins, DNase-free Proteinase K was added to a final concentration of 20 μg/ml followed by incubation at 37 °C for 30 min in a water bath with intermittent shaking. The solution was removed and the root samples washed thrice with 1x PBS at room temperature.

TUNEL assay. For *in situ* TUNEL assay Click-IT-Plus Tunel Assay kit (Thermo Fisher Scientific) was used according to manufacturer recommendations. Briefly, root samples were cut approximately 1 cm length from the root tip using sharp razor blades. The cut root tips were collected in microcentrifuge tubes (2 ml), one tube for each sample. TUNEL reaction was performed in these tubes. 110 μl of TUNEL reaction mix [16] was added to each of the microcentrifuge tubes. The reaction was carried out at 37 °C for 1 h in the dark humid chamber. The microcentrifuge tubes were covered with aluminum foil to avoid exposure to light. The reaction was stopped by adding 1 ml of 2x saline-sodium citrate (2x SSC) buffer (pH = 7.0). 2x SSC solution was removed and root tips were stained by addition of 100 μl of 0.05 μg/ml DAPI (4′,6-diamidino-2-phenylindole) solution followed by incubation at room temperature for 10 min in dark humid chamber. DAPI solution was removed and washed thrice with 1x PBS. The root tips were mount onto slides with ProLong® Gold Antifade reagent (Thermo Fisher Scientific, USA).

The samples were analyzed using a LSM 510 META confocal microscope (Carl Zeiss, Germany) with 10x objective and 0.22 NA. Imaging was performed using 405 nm laser for the visualization of DAPI nuclear staining and 488 nm laser for the incorporated Alexa-488-12-dUTP. All images were captured using the same microscope settings.

Individual cells in roots were identified with Fiji software. The Substract Background function was applied to each image with rolling ball radius of 25 pixels. Next, Li threshold was applied to the DAPI channel and used this as a mask for cell identification. Watershed function was applied before analysing particles with built-in tool. Particles were filtered by size ranging from 25 square micrometers. Individual cells identified with method described above were analyzed on mean intensity in green channel, which represent amount of DNA damage determined by TUNEL assay.

Nuclease activity assay. The activity of endonucleases was determined in solution *in vitro* using an assay based on the determination of the content of acid-soluble nucleotids formed under the action of DNases [17]. To determine the nuclease activity of the protein extract, meristems of the maize or pea seedlings roots were grinded in a porcelain mortar with the addition of a buffer containing 150 mM Tris-HCL (pH = 6.8), 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1 M dithiothreitol. The homogenate was centrifuged for 10 min at 12000 g (4 °C) and supernatant was used to evaluate the nuclease activity. Native and heat-denatured calf thymus DNA was used as a substrate. For the independent quantitative estimation of the «acidic» and «alkaline» DNases content we used modified assay [18]. The activity of alkaline DNases was evaluated in 100 ml of 5 mM Tris-HCl buffer (pH = 7.5) containing 5 mM $CaCl_2$, 5 mM $MgCl_2$ and 20 mg calf thymus DNA. The activity of «acidic» DNases was evaluated in 100 ml of 40 mM Na-acetate buffer (pH = 5.5) with 0.1 mM EDTA and 20 mg thymus DNA. For each assay, 5 μl of total protein extract from corresponding sample was added to the reaction. After addition of the protein extract, the reaction mixture was incubated at 37 °C for 1 h. The reaction was stopped by the addition of double volume of 0.5 M $HClO_4$, kept on ice for 30 min followed by precipitation of non-hydrolyzed DNA by centrifugation. Absorbance of the supernatant at 260 nm was measured by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). Final nuclease activity was adjusted for amount of total protein from added extract and measured as A_{260} units per μg of protein extract per hour.

Protein assay. Protein concentration in plant extracts was determined by modified linearized Bradford assay [19, 20], which uses 590/450 nm absorbance ratio to determine protein quantity. We prepared a series of standards of bovine serum albumin diluted in extraction buffer with different concentrations: 0 (blank = no protein), 250, 500, 750 and 1500 μg/ml. Protein extracts from root tissues under the herbicide treatment were diluted fivefold before measurements to maintain in the linear range of Bradford assay. We used 1:40 ratio of sample to Bradford reagent. After 5 min at room temperature, absorbance of each standard and unknown sample was measured at 590 nm and 450 nm by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). Linear dependency between protein concentration and 590/450 nm absorbance ratio was measured using LibreOffice Calc software for BSA standards, and derived linear regression formula was used to determine concentration of the unknown samples.

Statistical analysis. Statistical analysis of the results was performed using a Libre Office Calc software package. Each experiment was performed in 4 biological and 3 technical replicates and was reproduced inde-

pendently two times. The comparisons were carried out using the HSD Tukey test. The differences between the data were considered significant at $p < 0.05$. The results are presented as mean and standard errors ($x \pm$ SE).

Results and Discussion

Reliable marker of apoptosis-like PCD is the DNA degradation into nucleosomal units («DNA ladder»), since it indicates that PCD process is already at the irreversible stage [10]. At the final stage of PCD further hydrolysis of genomic DNA occurs (DNA fragmentation), which «strikes the last blow» to a cell. During this hydrolysis, the amount of free DNA ends increases that can be detected by the TUNEL assay. Due to the fact that DNA degradation into nucleosomal units is also possible during normal physiological aging of plants [13], all treatments have been compared with corresponding control.

Under the effect of propaquizafop ($5 \cdot 10^{-5}$ M), the increase of Alexa 488 fluorescence intensity in the maize cells was established at 4th day after treatment (DAT) (Fig. 1, *1, 2*; Fig. 2, *a, b*). This coincides visual with the appearance of necrosis in the maize roots meristems. Visually, at the 4th DAT all the seedling meristems were affected by necrosis. The treatment of pea roots with ALS-inhibiting herbicide also resulted in increase in Alexa 488 fluorescence intensity at 5th DAT (Fig. 1, *3, 4*; Fig. 2, *c, d*).

Endonucleases, including DNAses, play the major role among the enzymes actively involved in plant PCD by first performing the internucleosomal fragmentation of DNA, and then its final degradation. Up to 30 endonucleases associated with PCD have been identified in plants. Plants have two major classes of endonucleases active to dsDNA — Zn^{2+}-dependent («acidic») and Ca^{2+} or Mg^{2+}-dependent («alkaline»). Both these endonuclease classes are involved in DNA hydrolysis during PCD [21—23]. Nucleases are capable of hydrolysis of both native, and denatured

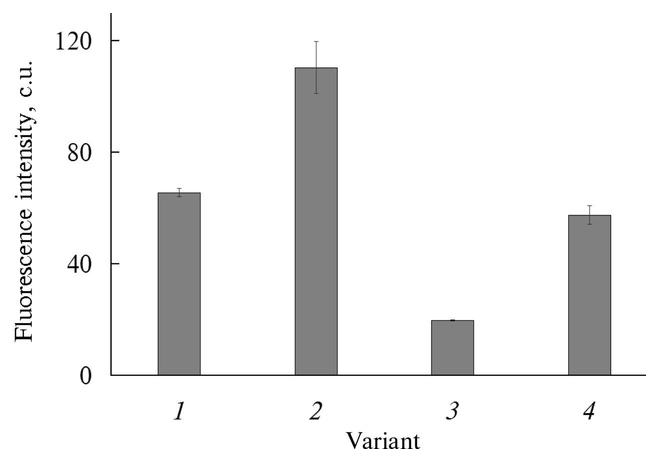

Fig. 1. Fluorescence of the root cells under the effect of herbicides, obtained by TUNEL assay:

1, 2 — maize tissue, 4th day after treatment, *1* — control, *2* — propaquizafop ($5 \cdot 10^{-5}$ M), *3, 4* — pea tissue, 5th day after treatment, *3* — control, *4* — tribenuron-methyl ($5 \cdot 10^{-5}$ M). The results on the diagram show fluorescence intensity average value (conventional units) per cells number in the sample

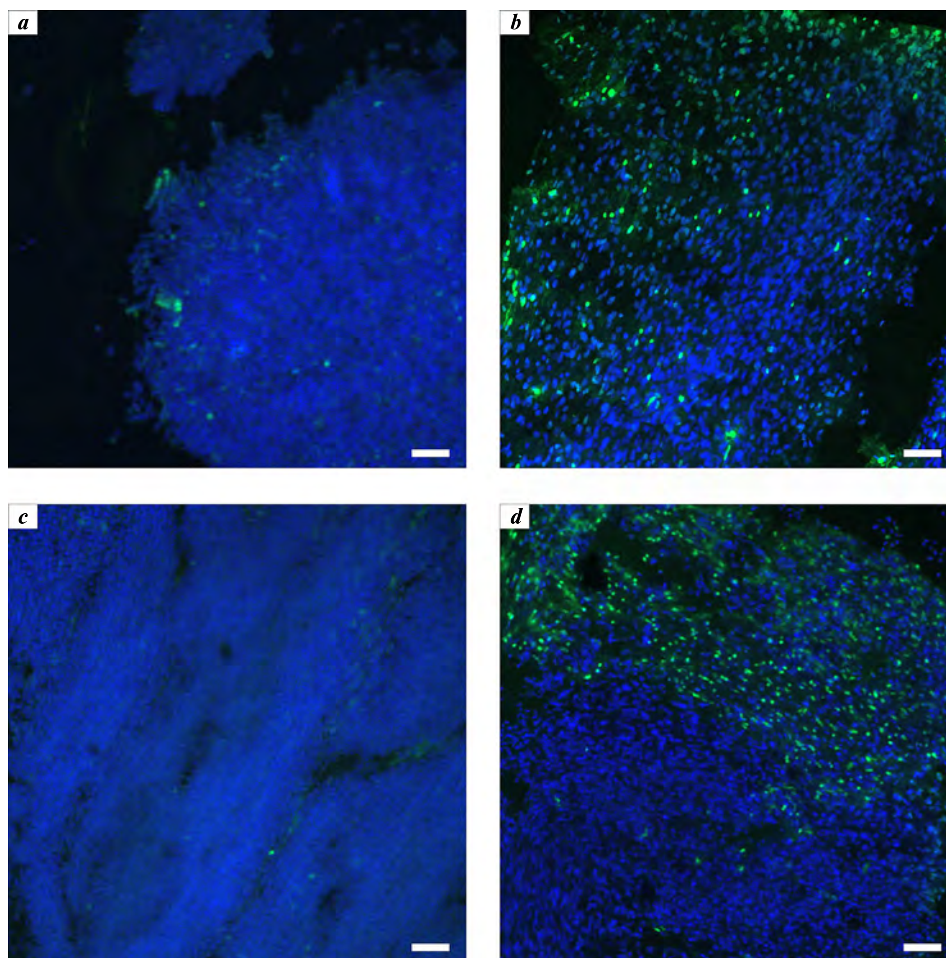

Fig. 2. Fluorescence (green spots) of the root cells under the effect of herbicides, obtained by TUNEL assay:

a, b — maize tissue, 4th day after treatment, *a* — control, *b* — propaquizafop ($5 \cdot 10^{-5}$ M), *c, d* — pea tissue, 5th day after treatment, *c* — control, *d* — tribenuron-methyl ($5 \cdot 10^{-5}$ M)

DNA. However, it is obvious that in PCD the degradation of DNA must occur due to the activation of nucleases, which substrate is native dsDNA.

Endogenous nuclease activities in root meristems of maize seedlings under the effect of ACCase-inhibiting herbicide propaquizafop, and pea seedlings under the effect of ALS-inhibiting herbicide tribenuron-methyl are shown in Fig. 3 and Fig. 4, respectively. Under the effect of both herbicides on the 3rd day, the total nuclease activity on native DNA in the root meristems of control plants is higher than in seedlings treated with herbicides (Fig. 2, *a*; Fig. 3, *a*). However, on the 4th day after treatment with propaquizafop, the total nuclease activity on native DNA in the treated plants significantly exceeded the activity in the control variant (Fig. 3, *a*). This is in accordance with the results from the TUNEL assay showing the DNA degradation on the 4th day after herbicide treatment. Similar increase in total nuclease activity on native DNA was observed in meristems of pea seedlings on the 5th day after tribenuron-methyl treatment

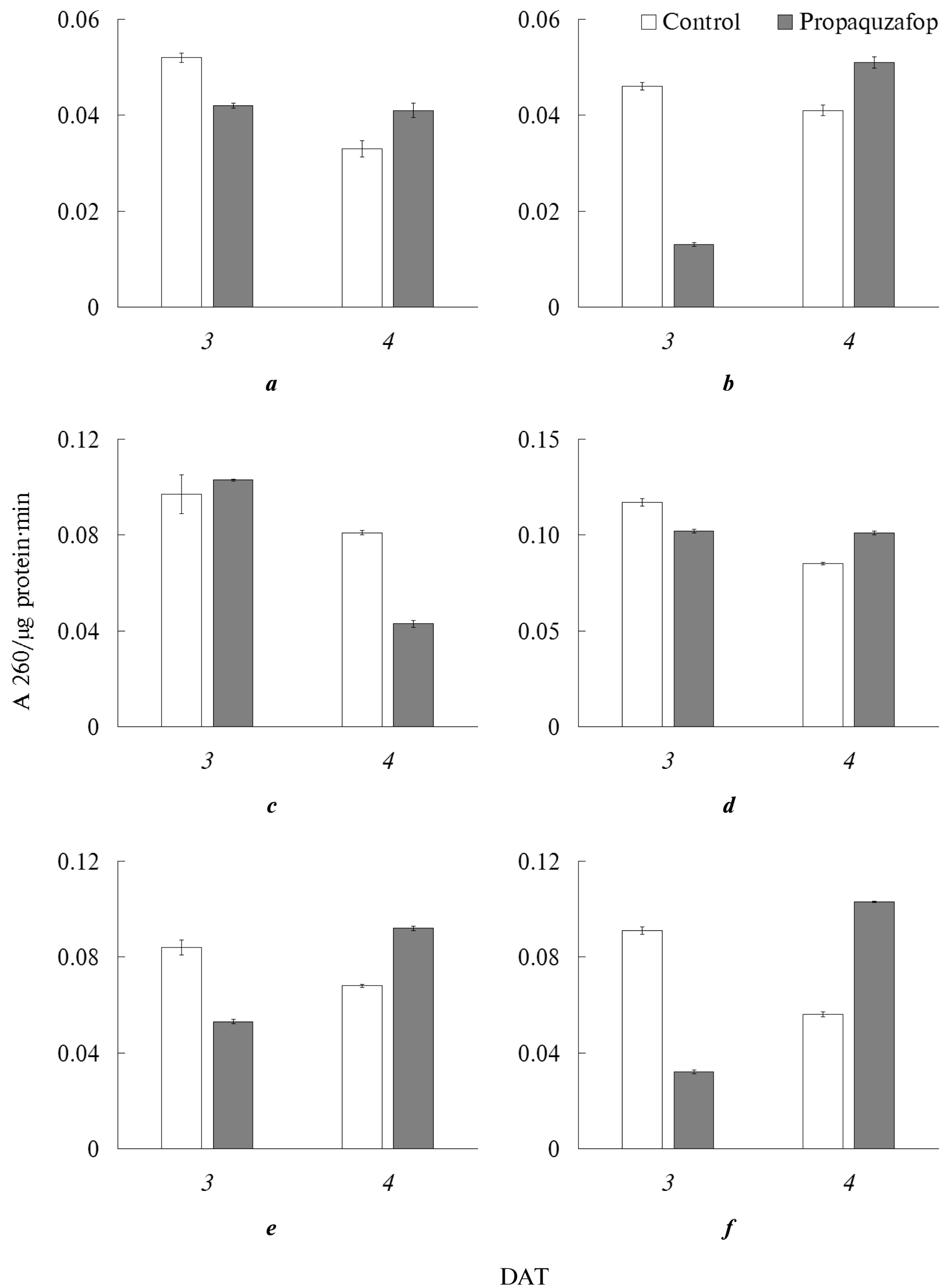

Fig. 3. Nuclease activity (A_{260}/μg protein·min) in the maize root cells under the effect of herbicide propaquzafop ($5 \cdot 10^{-5}$ M) on the 3rd and 4th day after treatment (DAT):

a, b — neutral buffer; *c, d* — alkaline buffer; *e, f* — acidic buffer; *a, c, e* — native DNA; *b, d, f* — denatured DNA

(Fig. 4, *a*), when DNA degradation occurred in these cells according to the TUNEL assay. In the pea cells under the effect of tribenuron-methyl, activity on native DNA of both alkaline and acidic nucleases was simultaneously increased (Fig. 4, *c, e*). At the same time the increase in general nuclease activity in maize cells was caused only by an increase in the acti-

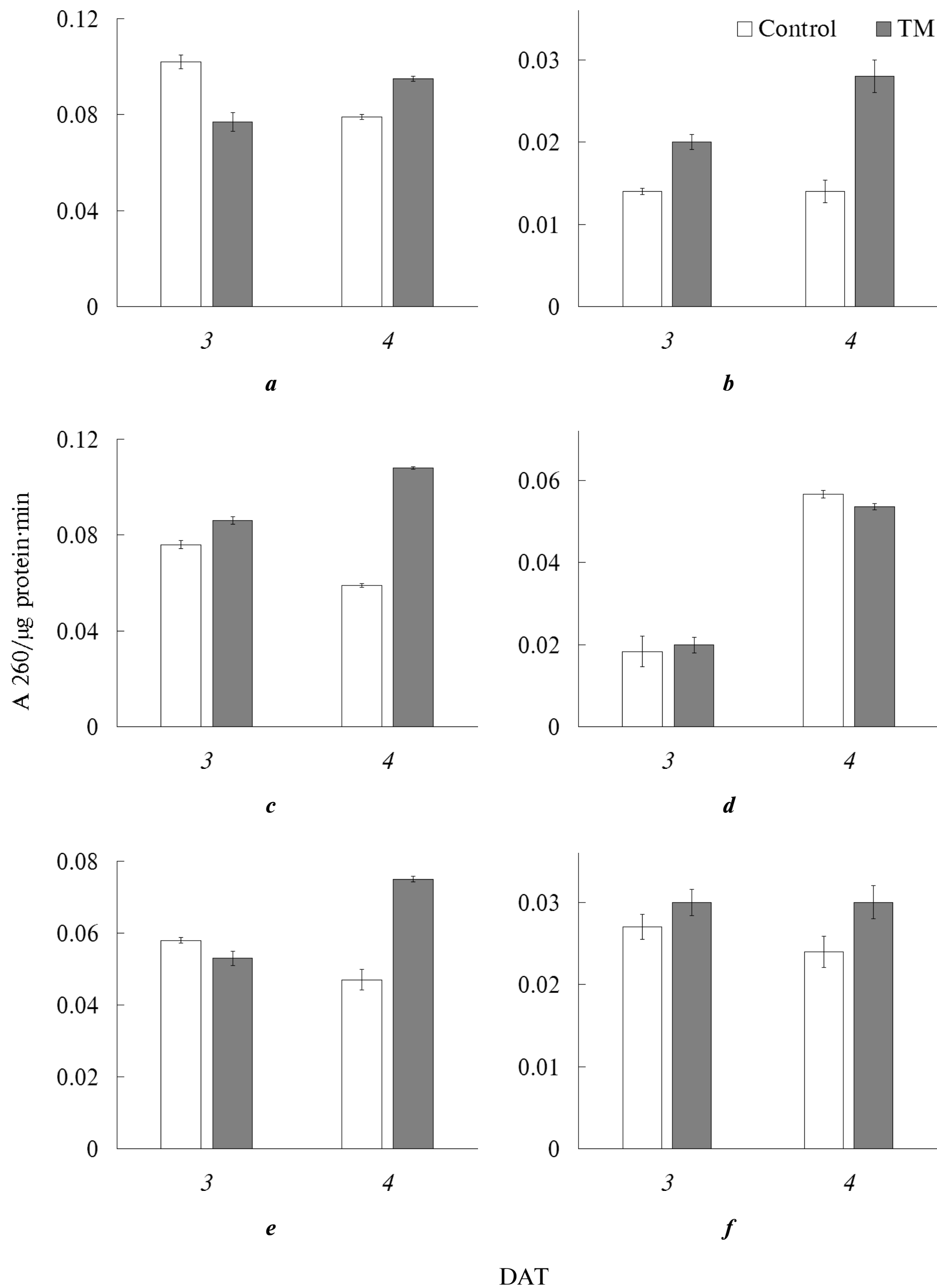

Fig. 4. Nuclease activity (A_{260}/μg protein·min) in the pea root cells under the effect of herbicide tribenuron-methyl (TM, $5 \cdot 10^{-5}$ M) on the 3rd and 5th day after treatment (DAT): *a, b* — neutral buffer; *c, d* — alkaline buffer; *e, f* — acidic buffer; *a, c, e* — native DNA; *b, d, f* — denatured DNA. Error bars denote standard error

vity of acidic nucleases (Fig. 3, *e*). Simultaneous increase in the activity of «alkaline» and «acidic» nucleases in the maize cells under propaquizafop effect was observed only on denatured DNA (Fig. 3, *d, f*).

In plants, the activation of PCD is required for physiological growth and development, and also is a part of the protective response to biotic and

abiotic stressors [13, 24]. PCD can be subdivided into three stages: signaling stage, execution stage, and dismantling stage. It is important to emphasize that during early stages PCD is reversible, and only upon entry into the dismantling stage cell death becomes irreversible. A distinct feature of the last stage is DNA fragmentation which is caused by activation of endonucleases [24]. Our study shows that under the effect of herbicides ACC and ALS inhibitors at the timepoint of DNA fragmentation the total nuclease activity in plant cells is increased in comparison with the corresponding controls. However, under the action of ALS inhibitor, the increase in the total nuclease activity was associated with the elevated activity of both «acidic» and «alkaline» nucleases, while under the action of ACC inhibitor, only higher «acidic» nuclease activity was detected. Such differences may be due to the distinction of these herbicides mode of action and, accordingly, to the differences in the mechanisms of PCD initiation.

A universal feature of apoptotic-like PCD in different types of organisms is the participation of ROS in the induction of this process [25]. Therefore, the first evidence for the involvement of PCD in induced pathogenesis was obtained for the herbicides which phytotoxicity is associated with disorganization of photosynthesis and mediated by ROS formation [3—5]. The pathogenesis of ACCase-inhibiting herbicides also includes the ROS overproduction [26—28]. It was shown, that the aging of coleoptiles, which is considered as a classic example of apoptosis-like PCD, is accompanied by increase in ROS content and activity of «acidic» nucleases [29]. Therefore, it can be assumed, that the activation of «acidic» nucleases at the terminal stage of PCD is connected with the participation of ROS in the initiation of this process under the action of ACC-inhibiting herbicides. An increase in ROS content also contributes to the development of the phytotoxic action of ALS-inhibiting herbicides, but this contribution is secondary to the final toxic effect [6]. This is probably due to the fact that source of ROS generation induced by ALS-inhibiting herbicides differs from one that caused by the action of ACC inhibitors. The investigation of ROS accumulation dynamics and NADPH oxidase activity after treatment by ACC-inhibiting herbicide showed that the NADPH oxidase activation is responsible for ROS formation only at the initial stage of a phytotoxic effect [30]. Thus under the effect of ACC-inhibiting herbicides ROS are apparently formed as a result of disturbances in the plastid electron transport chains caused by the blocking of fatty acid synthesis. At the same time, the study of NADPH oxidase inhibitor and calcium antagonists influence on the effect of ACC-and ALS-inhibiting herbicides showed that ROS formation induced by ALS inhibitor is more related to the NADPH oxidase activation [14]. It is known, that one of the consequences of ALS-inhibiting herbicide action is blocking DNA synthesis [31]. It is also known, that DNA fragmentation during PCD induced by alcaloid camptothecin, which inhibits DNA synthesis, occurs due to the activation of «alkaline» Ca^{2+}- or Mg^{2+}-dependent nucleases [32]. It is reasonable to conclude that the mechanism of PCD initiation caused by the action of ALS-inhibiting herbicides may be associated with DNA synthesis impairments.

Uncovering the mechanisms of PCD induced by the action of various herbicides can be of great importance for the development of methods for increasing their selective phytotoxicity. At present, the modification of the

herbicides phytotoxic effect can be achieved by influencing the factors responsible for the initiation of PCD. In particular, the efficacy of some herbicides depends on the antioxidant protection system state, and ROS production rate [33, 34]. So, it had been shown that the reduction of ACC-inhibiting herbicides phytotoxicity on grass weeds under drought conditions, and in tank mixtures explained by activation of antioxidant system by drought [28]. The usage of a superoxide dismutase inhibitor leads to decrease antagonism in mixtures of the ACC inhibitor fenoxaprop-*p*-ethyl with the ALS inhibitor amidosulfuron [27]. Therefore, to avoid antagonism in the mixtures with ACC inhibitors, it is necessary to use herbicides with high prooxidant activity [26, 35]. From another hand, antioxidant agents can be used to protect crops by reducing high oxidation in plant cells caused by herbicides [36]. For example, the action of NO, which in low concentration exhibits antioxidant activity, protects crops from the negative effect of different herbicides [37]. At the same time, high concentrations of NO as well as ROS can induce PCD [38]. So, it had been shown the increase of herbicide fenoxaprop-*p*-ethyl action by the donor of NO sodium nitroprusside [39].

Modifications in the state of antioxidant system in weed plants can cause the appearance of not target site related resistance (NTSR) to herbicides [40—45]. It is possible, that NTSR may be associated not only with changes in antioxidant activity and ROS production, but also with modification of other stages of PCD, since there is an undoubted relationship between different stage of PCD. So *Arabidopsis* double mutant with knockdown of antioxidant enzymes catalase and ascorbate peroxidase demonstrates the increase resistance to oxidative stress, which is apparently associated with an increase in the activity of antiapoptotic proteins [46].

Our investigation showed that DNA degradation caused by herbicides ACC and ALS inhibitors is associated with activation of endogenous nucleases. This result is additive confirmation of PCD participation in pathogenesis induced by these herbicides. Considering the literature data on the pathogenesis induced by herbicides of other classes [3—5], it can be concluded, that regardless of the specific site of action, the pathogenesis induced by herbicides occurs with the participation of PCD. The significance of this statement is that it emphasizes the fact that herbicide-induced pathogenesis is a complex, multi-stage, active process. Features of the course of pathogenesis can determine the dependence of herbicides action on environmental factors, and the nature of the interaction effects in herbicides mixtures. Mutational changes in the mechanisms that control individual stages of pathogenesis may be responsible for the emergence of NTSR herbicide resistance. At the same time, the discovery of the nature of these mechanisms opens up new possibilities for regulating the selective phytotoxicity of herbicides by physiologically active substances and genetically engineered manipulations.

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ФРАГМЕНТАЦІЯ ДНК ТА АКТИВНІСТЬ ЕНДОНУКЛЕАЗ ЗА ДІЇ ГЕРБІЦИДІВ ІНГІБІТОРІВ АЦЕТОЛАКТАТСИНТАЗИ ТА АЦЕТИЛ-КоА-КАРБОКСИЛАЗИ

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Метою дослідження була перевірка гіпотези про те, що патогенез, індукований у чутливих рослин гербіцидами інгібіторами ацетолактатсинтази (АЛС) та ацетил-КоА-

карбоксилази (АКК), є активним процесом, який включає програмовану загибель клітин (ПЗК). Надійним маркером ПЗК є фрагментація ДНК, яка здійснюється ендогенними нуклеазами. Кореневі меристеми чутливих до гербіцидів рослин були проаналізовані за допомогою аналізу TUNEL для оцінки фрагментації ДНК. Показано, що найвищий рівень фрагментації ДНК у клітинах кореневої меристеми при патогенезі, індукованому в проростках кукурудзи гербіцидом інгібітором АКК пропаквізафопом, та в проростках гороху гербіцидом інгібітором АЛС трибенуронметилом, пов'язаний з підвищенням загальної нуклеазної активності в лізатах клітин порівняно з відповідним контролем. У відповідь на застосування трибенуронметилу рівень загальної нуклеазної активності підвищувався внаслідок високої активності як Zn^{2+}-залежних («кислих»), так і Ca^{2+}/Mg^{2+}-залежних («лужних») нуклеаз, тоді як у випадку пропаквізафопу підвищена активність виявлена лише для Zn^{2+}-залежних нуклеаз. Ці відмінності можуть бути зумовлені різними механізмами ініціації ПЗК залежно від механізмів дії гербіцидів. Той факт, що за дії гербіцидів інгібіторів АЛС та АКК у клітинах меристем коренів проростків чутливих до цих гербіцидів видів рослин відбувається фрагментація ДНК, яка фіксується методом TUNEL, і ця фрагментація пов'язана з підвищеною активністю ендогенних нуклеаз, є доказом участі ПЗК в індукованому патогенезі. Значення цього твердження полягає у наголошенні, що індукований гербіцидами патогенез є складним, багатоетапним, активним процесом. Розкриття природи механізмів окремих стадій патогенезу відкриває нові можливості для регулювання вибірної фітотоксичності гербіцидів за допомогою фізіологічно активних речовин і генно-інженерних маніпуляцій.

Ключові слова: фрагментація ДНК, ендонуклеази, гербіциди, програмована загибель клітин, пропаквізафоп, трибенуронметил.