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TOWARDS A PARADIGM SHIFT IN THE FUCTIONS OF BIOLOGICALLY ACTIVE AGENTS: HERBICIDE-MEDIATED HORMESIS

One of the most important points to which I pay particularly close attention in this paper is the action of biologically active chemicals (BACs) in the living things. Theoretically, the action of BACs manifests itself in four stages, which are determined by the doses from the smallest to the largest: absence of visible effects, stimulation of biochemical and growth processes, inhibition of growth processes and death of the living things. Today, scientific research is mainly focused on the second stage of BACs action. At the same time, using of herbicides (killers of unwanted plants) in agriculture give evidens that they stimulate the growth of beneficial plants. The paradigm of only the stimulating action of BACs should be replaced by the paradigm of events at all four stages of BACs action in living organisms. The article proposes the mechanisms of events at all four stages of BACs action. The main focus is on redox reactions. Mechanisms of transformation of inactive BACs into reactive agents in endogenous redox reactions are described. This article also aims to focus the discussion on the analysis of the diversity of mechanisms underlying the action of natural and synthetic chemical agents.

Keywords: BACs, herbicides, hormesis, receptors, redox.

I. Herbicide hormetic effects

The lethal, inhibitory and stimulating effects of various chemicals have been known for a long time. This phenomenon has been known since the time of Paracelsus, who stated: "What is there that is not a poison? All things are poison, and nothing is without poison. Solely the dose determines that a thing is not a poison". The term a poison currently is also used as the biological active chemicals (BACs). For most BACs the stimulatory, inhibitory and lethal action is well known. Here I will focus on the well-known class of BACs such as herbicides. For herbicides, the most studied is the plant growth stimulating effect at a low dose and lethal at a high dose, which is known as hormesis.

Herbicides are a class of pesticides widely used by farmers to control unwanted vegetation. The use of herbicides is based on their lethal effect on undesirable plants for agriculture. Paradoxically, the practical use of herbicides in the field conditions has shown their growth-stimulating effect on beneficial plants. This phenomenon in further research was used in practical plant cultivation.

Further studies established the hormetic effect of sub-lethal doses for a number of chemicals: 2,4-D, metribuzin [1], 2,4-D, picloram, cloramben, dicamba [2], atrazine [3], diquat, glyphosate, metsulfuron, terbuthylazin [4], glyphosate [5], mercuric chloride, chromium chloride, ginseng, cadmium chloride, 2,4-D, atrazine [6], aminopyralid, barban, chloramben, bromoxynil, diuron, MCPA, quinclorac, simazine, terbacil [7].

Based on our earlier publications [8], proposed mechanism for BACs action in different concentrations is shown in Figure 1.

2. Dose-effect paradox

The dose-effect phenomenon in the action of BACs in various living systems is well known: increasing the BACs dose exposition is not always accompanied by increasing in the biological effect. Consequently, the same conclusions made on different objects, for example, on wild and mutant phenotypes, can differ significantly. A priori, chemical metabolism/biotransformation in different living systems can differ significantly not only because they have more or fewer transcribed genes, but also because chemical metabolism/biotransformation has different directions.

First of all, different living organisms have different defense systems (penetration rate exogenous chemicals into cell compartments, destruction, metabolization, neutralization and destruction of chemically active substances, etc.). For example, the resistance of monocotyledonous or dicotyledonous plants to the action of the 2,4-D herbicide may be as follows: relatively low concentrations of 2,4-D are lethal for dicotyledonous plants; moderate concentrations of the 2,4- D cause the death of both dicotyledonous and monocotyledonous plants. Also 2,4-D and tordone are used at relatively low concentrations in plant tissue culture as growth promoters [9-11]. Following the logic of the signaling theory of receptors, these substances in low concentrations should also use receptors to initiate growth. In this regard, the following questions arise. How many 2,4-D molecules are needed to initiate RNA transcription? Why is not observed transcription at high concentrations of 2,4-D in the cell that is accompanied by cell death? What happens to the receptors in this case?

Moreover, physical factors such as ultraviolet radiation can also affect plant metabolism and development [12, 13]. Are any receptors involved in these processes?

Thus, literature data indicate that studies of BAC action are focused on the transcription processes at low concentrations. At the same time, the participation of receptors in the mechanisms of growth inhibition (at relatively average concentrations) and lethal action (at high concentrations) is not considered.

There is no answer what is more important for gene transcription the protein or the BAC? It is also unknown how the BAC recognizes its “own” protein-receptor, and how proteins interact with each other in such structure. What is the structure of the newly formed molecule: is it a new molecule or a certain ligand? It is difficult to predict the stability of such formation if there is no information about the nature of the bond between the protein and the BAC, as well as between proteins. Furthermore, the interaction of RNA polymerase with such ligands is not yet fully understood.

3. Transcription activation by hormetic mechanisms of action

An important factor in herbicide hormesis is gene transcription. It is necessary to find out whether the stimulating effect of herbicides is associated with gene transcription. Is it possible to stimulate plant growth without the active participation of the nucleus, that is, without activating RNA synthesis? It is unlikely that the activation of growth processes is supported by the activity of existing enzymes at the onset of herbicide action. Therefore, two options are theoretically possible: increasing the rate of RNA synthesis on already open genes, or initiating the transcription of new genes. In the second option, the question of receptors will be inevitable. Today, the question of receptors for the destructive action of herbicides is replaced by the action of different classes of herbicides on individual cell structures, which is identified at different times after the herbicide enters the plant cell. At the same time, there is no identification of both the starting chemical reaction of herbicides and the starting cellular structure. Unfortunately, data on the effect of herbicides on RNA synthesis are sparse.

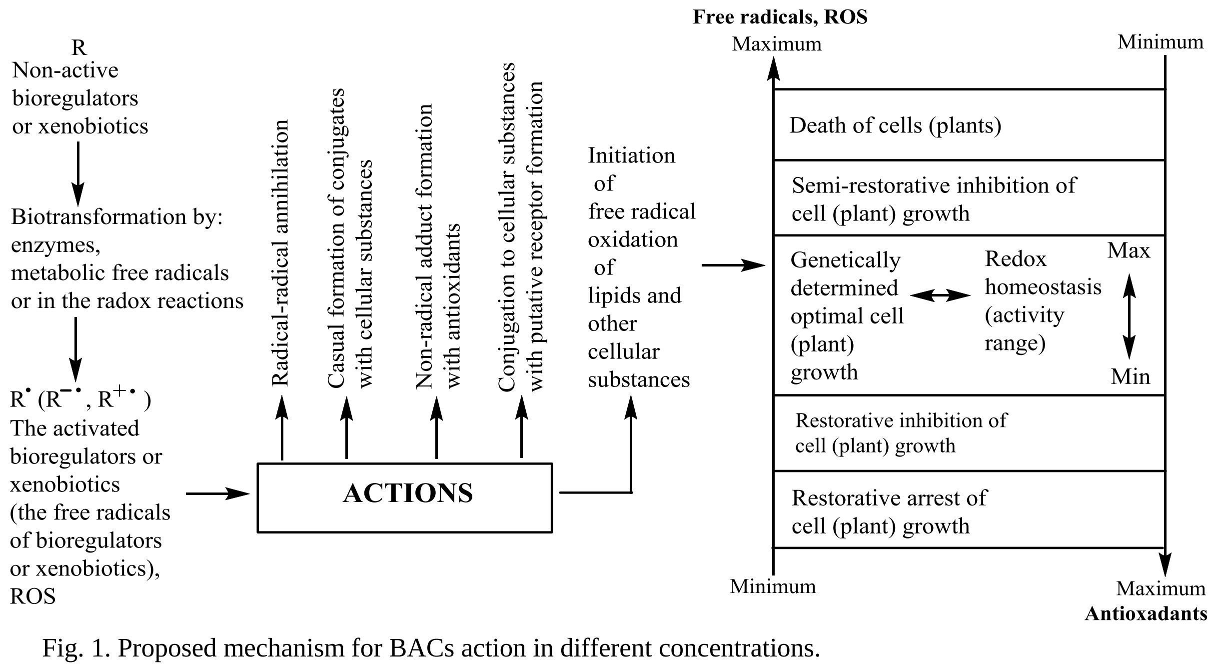

Fig. 1. Proposed mechanism for BACs action in different concentrations.

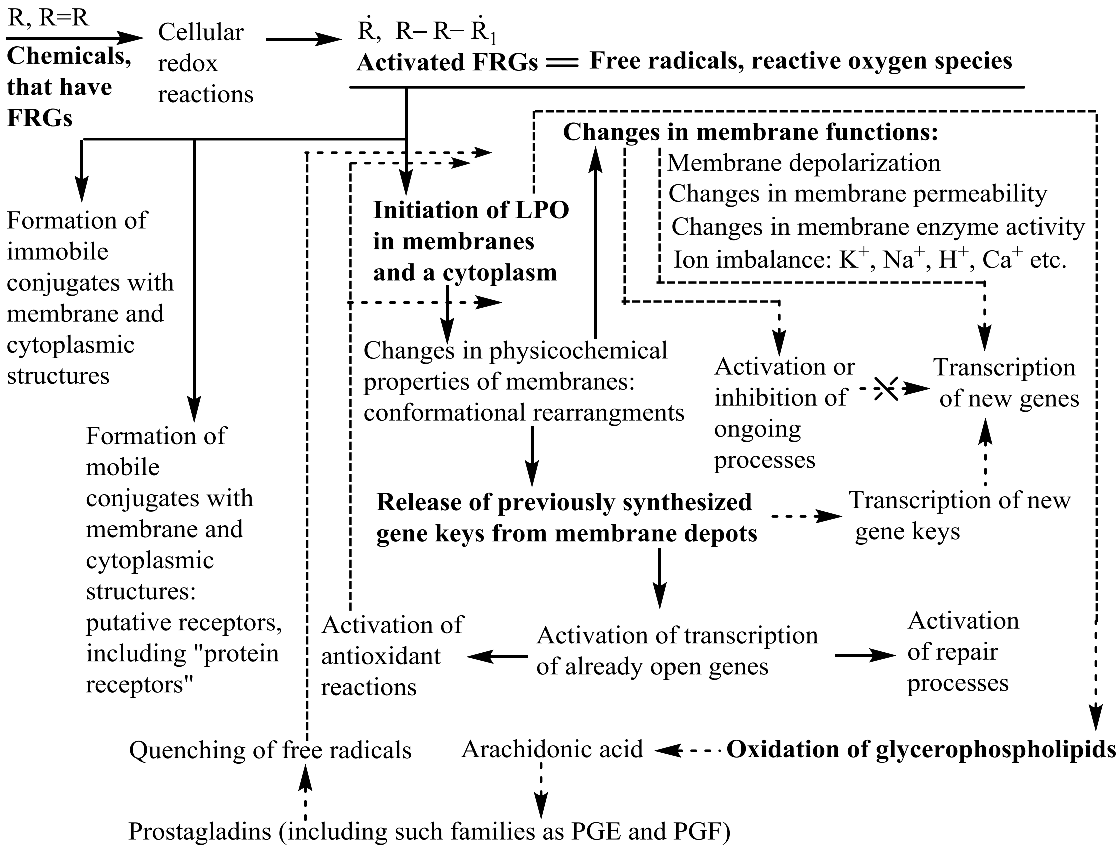

Fig. 2. Proposed mechanism for the primary stage actions of BACs in plants.

It is shown that herbicide quinclorac up-regulated P450 and GST genes in the plants of rice [14]. Application of herbicide (iodosulfuron+mesosulfuron) enhanced CYP gene expression in the grass weed *Lolium sp* [15]. Zhang Z-W et al. [16] found that plant cell RNA increased in Arabidopsis mutants under influence of herbicide norflurazon and amitrole. The expression of miRNAs (gra-miR7487c, gma-miR396f, gramiR8759, osa-miR395f, ath-miR847) was increased after herbicide application in both susceptible and resistant biotypes of *Echinochloa crus-galli* (L.) P. Beauv [17].

Based on our previously published data [8], the scenario of the events described above is presented in Figure 2.

4. Signal transduction: biologically active substances can act with or without the participation of membrane receptors

Key events of biochemical processes in the cells of living beings occur due to the process of "signal transduction". The central dogma of signal transduction is the theory of protein receptors. This section does not cover this theory. I have not found experimental confirmation of this theory in the literature *in vitro*. It does not provide answers to a number of fundamental questions. How the so-called ligand can stimulate, inhibit or cause death of the living organism, depending on the concentration in the cell? Moreover, BACs may not show the biological effect at some low concentration. How do physical factors, such as temperature, magnetic and electric fields, X-rays and ultraviolet radiation, work from the receptor theory point of view? In addition, physical and chemical factors can affect only physiological processes without changing genomic processes (for example, transcription or replication), or simultaneously affect both processes. There is also no clear answer to the fundamental question of such factor in the biology of the action of BACs as "cross-talk", when chemical substances of different structures can cause the similar biological response in living systems, depending on the effective concentration.

One hormone can cause more than one physiological effect [18]. A similar effect in the terminology of cross-talk is also known for phytohormones [19]. Since gene transcription is the selective process, therefore, phytohormones of different steric structures cannot directly initiate the transcription of the same gene. It can be assumed that phytohormones of various structures induce the formation of certain mediators, identical in chemical action (not necessarily in structure), which initiate, for example, transcription of the one individual gene. Free radical structures can be such secondary intermediaries.

The number of protein receptors described in the literature is steadily increasing. If the list of physical factors is limited, then the number of chemical factors that influence on living systems is not limited. It is possible that the number of natural BACs will eventually reach the ceiling, but the number of synthetic BACs is not limited. Therefore, the number of BACs will many times exceed the number of possible receptor proteins. In the future, a situation is possible when dozens or hundreds of BACs will be identified with the same receptor protein, and this negates the selectivity of gene functioning in living systems.

If there are dozens of biologically active substances in the cells of plants or animals, how is the sequence of receptor complex formation? Why does the simultaneous formation of receptor complexes by all available BACs in the cell not occur? Why are there thousands of RNA polymerases in the cells of living things, but RNA transcription, according to electron microscopic photographs, is usually observed in 1-3 nucleoli of the cell nucleus?

In the theory of protein receptors, there are no specific (at the level of elementary chemical reactions) mechanisms about how the receptor complex assembled on the plasmalemma finds the desired RNA polymerase, and then the desired chromosome, and the desired gene in chromosome. Moreover, what biological (or chemical) law can explain why such the small molecule as ethylene (or nitric oxide), combine with the much larger (in size) protein, and then push between the cytoplasm molecules to the nucleus in searching of the “necessary” gene among thousands of genes. What “magic force” makes ethylene, after combining with the protein, move to the cell nucleus? How does ethylene know that there are structures in the nucleus that “need” its services? How are labeled genes and how do they differ from each other?

In addition, the protein BACs in living systems, with a few exceptions, are represented by cyclic peptides (some of them consist of more than 100 amino acids). The question arises. What is the nature of the chemical bonds between two proteins: a biologically active protein and a receptor protein? How does the protein complex consisting of the receptor protein and the biologically active peptide find the corresponding gene in the chromosome that is also coated with the protein coat?

The supposed mechanism of living system functioning under the influence of physical and chemical factors is shown in Fig. 3. The simplified model shows only two variants of possible events. In the first variant, the action of chemical and physical factors can be accompanied by changes in membrane functions (primarily in plasmalemma) without changing the processes of DNA transcription. In this case, physiological and biochemical processes in the cell can be both stimulated and inhibited. In the second variant, the change in physiological and biochemical processes is accompanied by both the intensification of the ongoing and the initiation of new transcription processes. First of all, the activation of protective (antioxidant) processes is observed.

In the plasmalemma of the cell, as the front line of defense, unsaturated fatty acids are concentrated, which are the first interceptors of oxidants (free radicals). In the redox destruction of fatty acids are formed: antioxidants, oxidants and neutral substances. Among neutral substances, there are potential BACs, which after activation (transformation to the free radicals) act as oxidants.

The formation of the ligand-receptor complex *in vivo* must be confirmed *in vitro*. If the protein is isolated from the membrane, representing the hypothetical receptor, then it should form the complex with the ligand *in vitro*. If such the complex did not form *in vitro*, then doubts arise about its formation *in vivo*.

For the new formed BACs, there is one peculiarity: the activation of these substances usually occurs outside the place of formation. This is especially important, for example, in the processes of elimination of foreign substances, including pathogenic organisms. One of the principles of getting rid of foreign living beings is the activation of oxidative (free radical processes) in the body of the foreign being.

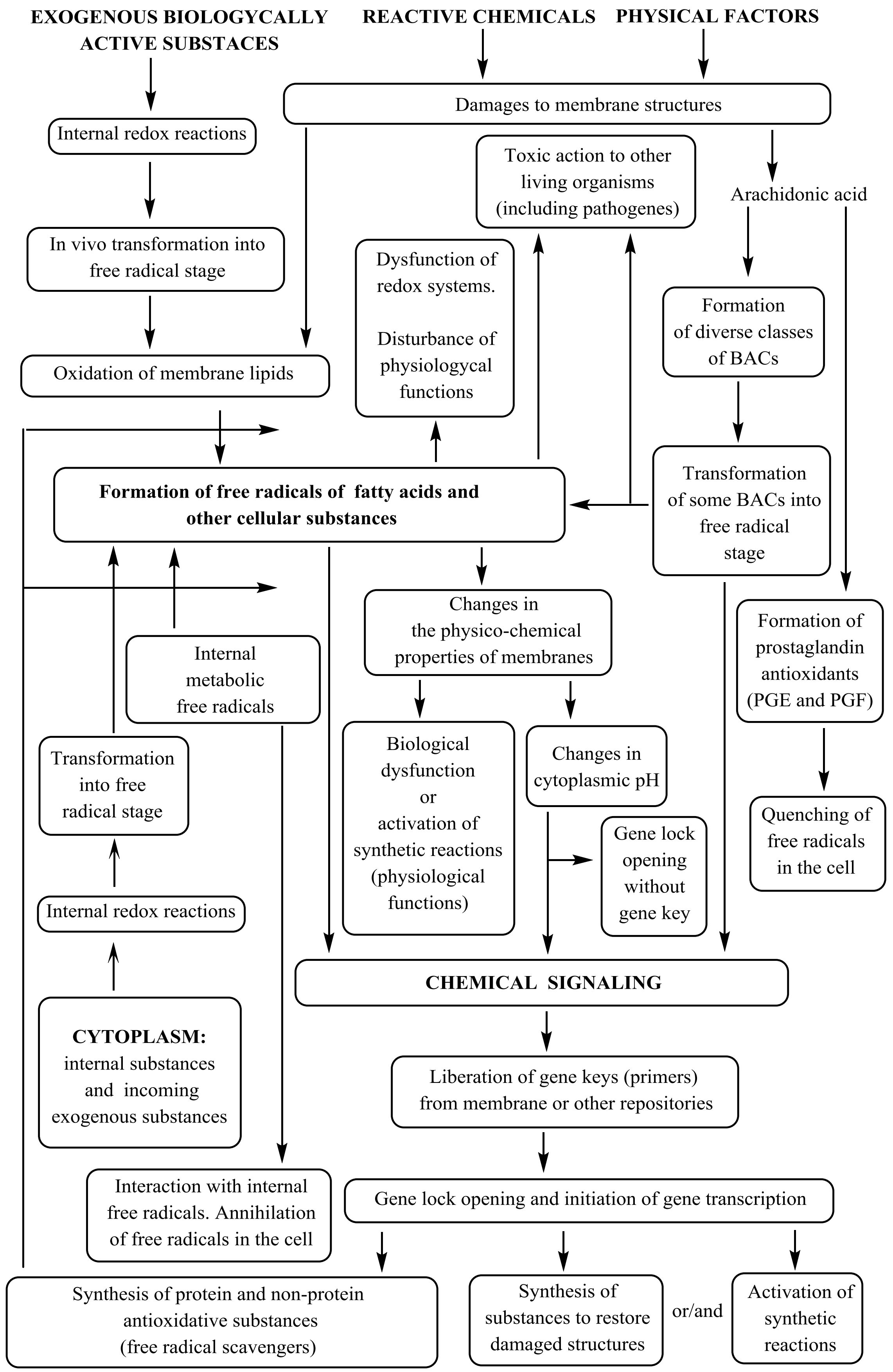

Fig. 3. A hypothetical scheme describing chemical signaling in the living systems.

Conclusions

The fundamental principle of action of physical and chemical factors, there may be changes in the functioning of redox systems. As you can see from the Fig. 3, both physical and chemical factors act primarily on membrane structures. In this case, the primary effect of physical factors is focused on violations of the membrane structure, followed by the oxidation of fatty acids of membrane lipids. Exogenous BACs can be activated both in membranes and within cells, i. e. in the cytoplasm. The transformation of BACs to the free radicals is possible with the participation of cytochromes (in the area of membrane structures) and endogenous metabolic free radicals.

The action of physical and chemical factors can only cause the change in the physicochemical properties of cell membranes and, accordingly, enzymes associated with the membranes. In this case, both activation and inhibition of physiological and biochemical processes are possible.

The activation or initiation of transcriptional processes follows the certain threshold of changes in physiological and biochemical reactions in the cell, i. e. permissible fluctuations from the genetic programs. It is possible that rapid changes in transcriptional processes can occur due to changes in the pH of the cytoplasm, i. e. without the participation of gene keys (primers), whereas the initiation of transcription of new specific genes requires the participation of gene keys. The primary response of the cell to the excess of the controlled level of oxidant agents and will be aimed at their neutralization.

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ДО ЗМІНИ ПАРАДИГМИ ФУНКЦІЙ БІОЛОГІЧНО АКТИВНИХ АГЕНТІВ: ОПОСЕРЕДКОВАНИЙ ГЕРБІЦИДАМИ ГОРМЕЗИС

Одним з найважливіших моментів, якому я приділяю особливу увагу в цій статті, є дія біологічно активних хімічних речовин (БАР) у живих істотах. Теоретично дія БАР проявляється чотирма стадіями, які детермінуються величиною доз від найменшої до найбільшої: відсутність видимих ефектів, стимулювання біохімічних і ростових процесів, гальмування ростових процесів і загибель живої структури. На сьогодні наукові дослідження зосереджені, головним чином, на другій стадії дії БАР у рослинних і тваринних організмах. У той же час, використання гербіцидів (кілерів небажаних рослин) в аграрному господарстві свідчить про їх стимулюючу дію на корисні рослини. Парадигма тільки стимулюючої дії БАР має бути замінена парадигмою подій на всіх чотирьох стадіях дії БАР у живих організмах. У статті запропоновано механізми подій на всіх чотирьох стадіях дії БАР. Основна увага зосереджена на редокс реакціях. Описано механізми трансформації неактивних БАР у реактивні агенти в ендогенних редокс реакціях. Ця стаття також має на меті зосередити дискусію на аналізі різноманітності механізмів, які лежать в основі дії природних і синтетичних хімічних агентів.

Ключові слова: БАР, гербіциди, гормезис, рецептори, редокс.