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An approach to the application of plant secondary metabolites in agriculture to improve food quality

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Abstract

The article presents a new methodology for improving food safety by regulating the distribution of pollutants (radionuclides, heavy metals, pesticides) in agricultural crops using secondary plant metabolites, in particular flavonoids. The results of using an improved method of supercritical fluid extraction of metabolites and theoretical analysis of experimental data from other scientists point the way to the creation of a new agricultural technology for obtaining environmentally friendly products, where the targeted use of flavonoids stimulates the natural defense mechanisms of plants. These mechanisms include the processes of synthesis of chelating proteins and enzymes in plants for the immobilization of pollutants in certain organs. Flavonoids have been shown to regulate antioxidant protection, complex formation and redistribution of metabolite transport as signaling molecules. This is the first study to combine supercritical fluid extraction and exogenous application of flavonoids to reduce pollutant translocation in crops. The purpose of this technology is to produce agricultural products that comply with international food safety standards and to enable the safe cultivation of crops on contaminated lands.

Keywords: *agricultural technology, secondary metabolites, radionuclides, pesticides*

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Подход к применению растительных вторичных метаболитов в сельском хозяйстве для улучшения качества пищевых продуктов

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Резюме

В статье представлена новая методология повышения безопасности пищевых продуктов путем регулирования распределения загрязняющих веществ (радионуклидов, тяжелых металлов, пестицидов) в сельскохозяйственных культурах с использованием вторичных растительных метаболитов, в частности флавоноидов. Результаты применения усовершенствованного метода сверхкритической флюидной экстракции метаболитов и теоретический анализ экспериментальных данных других ученых указывают путь к созданию новой агротехнологии получения экологически чистой продукции, в которой целенаправленное использование флавоноидов стимулирует естественные защитные механизмы растений. К этим механизмам относятся процессы синтеза хелатирующих белков и ферментов в растениях для иммобилизации загрязняющих веществ в определенных органах. Доказано, что флавоноиды регулируют антиоксидантную защиту, комплексообразование и перераспределение транспорта метаболитов как сигнальные молекулы. Это перспективное исследование, в котором сверхкритическая флюидная экстракция и экзогенное внесение флавоноидов сочетаются для снижения транслокации загрязняющих веществ в сельскохозяйственных культурах. Целью данной технологии является производство сельскохозяйственной продукции, соответствующей международным стандартам безопасности пищевых продуктов, и обеспечение безопасного выращивания сельскохозяйственных культур на загрязненных землях.

Ключевые слова: *агротехнология, вторичные метаболиты, радионуклиды, пестициды*

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Introduction

One of the most critical objectives of preventive medicine worldwide is ensuring a safe and high-quality food supply [1-4]. In this context, maintaining human health and preventing disease require rigorous food safety monitoring.

Due to the rapid growth of the global population and the increasing volume and diversity of food products, controlling their quality has become increasingly challenging. The problem is exacerbated by the shortage of arable land, necessitating the use of agricultural territories that do not fully meet regulatory standards. This compels the intensified chemicalization of soils through the application of fertilizers, herbicides, pesticides, growth stimulants, and other substances to achieve required soil characteristics. Consequently, this leads to soil contamination and the accumulation of harmful substances in plant and animal products consumed by humans [5-6]. Another agricultural challenge involves territories that are otherwise suitable for farming but are contaminated with various pollutants. Examples include lands contaminated with radionuclides [6]. The Chernobyl Nuclear Power Plant accident resulted in such contaminated areas across Belarus and Russian Federation. Consuming food produced on radionuclide-contaminated agricultural lands causes severe disruptions to the human health. The aforementioned issues underscore the urgent need to develop methods for ensuring the safety of agricultural products.

The accumulation of soil pollutants in plants often occurs through molecular mechanisms that may not overlap, leading to the evolution of diverse natural defense mechanisms in plants against specific substances. This necessitates repeated treatments of crops with artificial protective substances during a single growing season, making it extremely challenging to predict and prevent all potential accumulations. The relevance of this problem is further amplified by the fact that the quality of raw materials and food products – especially regarding radionuclide content – often fails to meet import requirements set by the Eurasian Union, and other trade partners of the Republic of Belarus. This significantly hinders and complicates agricultural exports [7].

To date, research efforts have focused on developing methods to reduce the accumulation of pollutants in agricultural plant.

Therefore, the objective of this study is to develop a novel agricultural technology that regulates the distribution of pollutants within crops through the targeted use of flavonoids and other secondary metabolites, with the goal of protecting economically significant plant parts and ensuring compliance with international food safety standards.

Scientific basis

The research presented in this article proposes a relatively new agrotechnological approach that utilizes our metabolite extraction technology [8-13]. It has been shown, that the metabolism and detoxification of heavy metals present particular challenges to biological systems. In plants, these responsible mechanisms include heavy metal-binding compounds such as the metallothioneins (MTs) and phytochelatins (PCs). MTs and PCs are different classes of cysteine-rich, heavy metal-binding peptide or polypeptide molecules. PCs are enzymatically synthesized peptides, while MTs are gene-encod-

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ed polypeptides [14]. It is shown that the metallothioneins are low-molecular-weight proteins which capable of covalently binding heavy metal ions due to the presence of many cysteine residues in their sequences. The predicted amino acid sequences of 19 metallothionein (7 from *Arabidopsis thaliana* and 12 from *Oryza sativa*) and their promoter sequences in silico in order to determine the potential regulatory cis-elements present in the promoters of metallothionein genes, from which it is possible to determine the putative functions of these genes. The PlantCARE and PLACE databases provided information about the putative regulatory elements in the metallothionein promoters. Metal response element sequences were found in the promoters of eleven *O. sativa* and two *Arabidopsis* metallothionein genes. Copper response elements were identified in both model plants, usually in many copies, particularly in *O. sativa*. Both the high cysteine content and the presence of metal response motifs in the promoters support the suggestion that metallothioneins play a key role in metal detoxification. The most common putative element in the analyzed promoters was CIRCADIAN, which was present in five *A. thaliana* and eight *O. sativa* sequences. The methyl jasmonate response sequence, root-specific expression element and drought response element were found only in *O. sativa* metallothioneins. Light and low temperature response elements, biotic and abiotic stress elements, an abscisic acid-responsive element and an ethylene-responsive element occur in selected metallothionein promoters of both species. A few promoters have putative organ- and cell-specific regulatory elements. The presence of many different motifs in the promoters of the *Arabidopsis* and *O. sativa* genes implies that metallothioneins are general stress response proteins with many important functions in plants, including regulation of their normal development and adaptation to changing environmental conditions [14].

The investigations into the effects of flavonoids on biochemical processes in plants provide a foundation for the successful implementation of the proposed methodology, as outlined in these works [15-18]. In particularity, the total flavonoid and anthocyanin content in different parts of *Matthiola odoratissima* plants decreased with increasing altitude [18].

The essence of our scientific approach, in contrast to previously employed approaches, is the development of new agrochemical techniques for the controlled distribution of contaminants in specific plant parts (root system, stem, leaves) that are not subsequently used in the production of human food or animal feed.

To implement this idea, we propose leveraging natural plant mechanism of plant adaptation and defense. We assumed specifically, chelating proteins cysteine-rich proteins named as metallothioneines, can bind to ions such as heavy metals so as radionuclides and can induce defense mechanisms for many stresses. It is shown that the high variability of the content of phenolic components in *Pentaphylloides fruticosa* different ecological and geographical conditions and during ontogenesis, expressed in the nature of distribution, the ratio of components and the change of the predominant compounds with the stability of the total content of the phenolic complex, confirms the participation of phenolic compounds in adaptation to environmental conditions. Authors found, that *P. fruticosa* plants growing under conditions of technogenic stress of different types acquire specific features: under conditions of industrial and transport influence, the content of phenolic compounds decreases and the seasonal dynamics of their accumulation changes; under conditions of radiation exposure (^{90}Sr, ^{137}Cs), the variability of the content of phenolic components (secondary metabolites) increases With the increase in radionuclide contamination, the content of individual phenolic components - isoquercitrin and kaempferol in the leaves of *P. fruticosa* increased, while ellagic acid, on the contrary, decreased [15, 16].

We proposed that the application of specific flavonoids could increase the adaptive effects of plants to radiation waste, and it can be evaluated by expression of metallothioneins, which are general stress response proteins with many important functions in plants, including regulation of their normal development so as adaptation to changing environmental conditions.

Additionally, they induce or activate enzymes involved in pesticide metabolism, such as cytochrome P_{450} and glucosyltransferases, which catalyze pollutants modification [19].

This approach aims not to disrupt biochemical processes in plants (which often severely alters their metabolism) but rather to harness a plant's inherent natural mechanisms to stimulate the accumulation of undesirable substances in predefined locations rather than throughout the entire plant body.

Research stages of technology

The purpose of this comprehensive multi-stage study is to develop a technology for controlling the distribution of pollutants within specific parts of agricultural plants, thereby enabling the production of high-quality food products. Our proposed agricultural technology is aimed at protecting agricultural crops from radionuclides and organic pollutants (pesticides, fertilizers, etc.) in high-risk regions such as Belarus and Russia. It combines agronomic, biochemical, and ecological approaches to ensure the safety of crop production in contaminated areas of

Belarus and Russia. Key tasks include crop selection, identifying optimal stimulants, conducting field trials, and analyzing biomaterials. Therefore, we plan to devote special attention to the selection of experimental sites, including a site with normal parameters for both radiation exposure and pesticides and other organic matter. Experimental sites must have a moderate radiation load (according to contamination maps) and be located on agricultural land. A site with a high radiation load that is not located on agricultural land will also be used. We will select experimental crops suitable for cultivation in the selected areas and economically viable for Belarus and Russia. Of particular importance is identifying the key biochemical mechanisms for protecting economically significant plant parts from elevated levels of radionuclides and various organic pollutants. From here on, we will use the term «protection of economically significant parts,» meaning that the essence of our approach is not to protect the plant as a whole, but to selectively protect (based on blocking the migration of pollutants) only those plant parts that are subsequently used for food and animal feed production. Crops must be adapted to the climate and soil conditions of the selected areas (e.g., Polesie in Belarus or the Chernozem Region in Russia), economically significant, and vulnerable to the accumulation of radionuclides (Cs-137, Sr-90) or organic pollutants (pesticides, genetic modified organisms (GMO)).

Priority is given to crops with clearly defined economically significant parts (fruits, grain, roots, and foliage for forage), where toxin migration is critical. We consider yield, rotation, and stress tolerance. We suggest that a key link may be identifying the most effective substances capable of stimulating various defense mechanisms in economically significant plant parts. For example: antioxidants (phenols, vitamins C/E) stimulate glutathione peroxidase, neutralizing reactive oxygen species and reducing oxidative stress in fruits; chelators (EDTA, citric acid) bind radionuclides in roots, preventing their translation into grain; they activate phytochelatins; growth hormones (salicylic acid) enhance the expression of detoxification genes, blocking their pathway to edible parts; microelements (K, Ca, Se) compete with toxins for absorption; they strengthen cell walls in leaves and fruits. Our focus is on substances that enhance the plant antioxidant system, chelation, and barrier functions of plant tissues to minimize pollutant migration (>30% reduction in target values). The following indicators are proposed to be used as analytical markers: markers of antioxidant protection (the concentration of glutathione and associated enzymes, the amount of oxidized membrane phospholipids, the concentration of natural antioxidants for a specific plant species), the degree of transfer of radionuclides into the economically important part of the plant, and the determination of the polymorphism of enzymes responsible for various protective mechanisms. These studies can be conducted using chromatographic and electrophoretic separation of proteins (enzymes, metallothioneins) of the corresponding plant and the formation of their qualitative and quantitative composition. There is data in the literature on the polymorphism of plant proteins as an adaptive mechanism [20-29]. Among the enzymes described are glutathione peroxidases and other enzymes of the oxidoreductase class, as well as enzymes of other classes. Low-molecular-weight organic compounds belonging to the flavonoid class can act as stimulators of their synthesis, and sometimes as coenzymes [30-44]. The expected results of this stage will be conclusions about the main mechanisms of plant defense against adverse environmental conditions, presented in the form of comparative maps of the quantitative content of chelating proteins and their polymorphism, and the identification of two or three universal stimulators adapted to soils (pH 5-7, sod-podzolic type).

The next step is to conduct a field experiment and obtain practical information on changes in the qualitative and quantitative composition of enzymes and gene expression stimulators in plant cells under the influence of various substances isolated from secondary metabolites. To achieve this goal, we plan to conduct pre-sowing seed treatment, sow and monitor treated plants in experimental plots, conduct biochemical and toxicological studies of the biological material to identify classes of substances capable of stimulating defense mechanisms at the molecular or genetic level, and establish the optimal method for stimulating the defense of economically significant plant parts. We also determine the degree of secondary metabolite polypotency relative to the studied defense mechanisms and identify optimal polypotent stimulators for areas with combined radiation and chemical pollution. In our experiment, we plan to use data on the quantity and types of secondary plant metabolites (flavonoids, terpenoids, alkaloids) to obtain the expected results. Flavonoids are the most interesting class. An effective method for their extraction is the high-tech method of supercritical fluid extraction. The proposed agricultural technology includes radiological and toxicological studies, specifically changes in the qualitative and quantitative composition of chelating proteins and enzymes responsible for protection against heavy metals and radioactive radiation. The correlation between the amount of radionuclides and the corresponding amount of chelating proteins has an adaptive basis. Confirmation of plant adaptation to increased radionuclide levels will be a change in the structure of chelating proteins. Proteomic analysis

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will identify proteoforms and expressed and inactive genes to assess the potential of alternative splicing as an adaptive defense mechanism. As mentioned, flavonoids can stimulate not only the sorption of radionuclides but also directly or indirectly inactivate pesticides. Therefore, it is necessary to determine the presence and extent of this polypotency of secondary metabolites beneficial for agriculture under experimental conditions. In the future, this will allow the selection of the optimal substance for protecting economically important plant parts. As a result, we expect to obtain data on changes in enzyme polymorphism and chelating proteins under the influence of various flavonoids. Studying polymorphic oxidoreductases will also allow us to determine the ability of plants to distribute organic pollutants in specific locations. Undoubtedly, an important step in implementing the proposed agricultural technology is obtaining practical information on the degree of toxicity of the obtained biomaterial for humans. Accordingly, we plan to conduct a toxicology experiment on laboratory mice; obtain mouse feed from plants grown in different regions and treated with various secondary metabolites; and conduct toxicology studies of mouse biomaterials using the results of field experiments. To confirm the safety of agricultural products obtained in areas contaminated with various pollutants, an experiment will be conducted on laboratory mice. The mice will be divided into three groups, each of which will receive food prepared from plant components collected in the experimental areas. The first group will receive feed prepared from plants grown in a relatively clean area; the second group will receive feed prepared from plants grown in a relatively contaminated area included in the agricultural fund. The third group will receive food from plants growing in contaminated areas that are not included in the agricultural fund. The effect of incoming radionuclides on mice will be determined using Q-FISH (quantitative fluorescence in situ hybridization), a micronucleus test, and bone marrow cell count analysis [45, 46]. Cytological studies will include both morphological and biochemical comparisons with the control. To confirm increased resistance to pesticides and other organic pollutants, measurements of key antioxidant defense parameters (glutathione levels and related enzymes) and blood biochemistry will be conducted. After identifying key correlations, the leading flavonoids that provide the greatest stimulation of both the antioxidant and chelating capacities of the selected plants will be identified. As a result of laboratory studies, we expect confirmation of low levels of contamination of the economically important plant parts by any pollutants, as well as a high antioxidant status, which may be beneficial for other biological systems when consumed as food.

In the future, we plan to identify optimal stimulants, confirm their effectiveness, and refine the key stages of our proposed agricultural technology. This will require conducting control sowing of the selected plants in experimental areas with appropriate treatment with secondary metabolites (flavonoids); refining and testing recommendations for pre-sowing treatment of plant material; and evaluating and confirming the multiple functionality of the selected flavonoid. Conduct toxicological studies of mouse biomaterial. The final stage of the experiment differs from previous studies not by selecting a single optimal immune system stimulant, but by confirming its effectiveness both as an antioxidant and as a stimulant of the expression of genes responsible for the synthesis of chelating proteins. The design of our study, both in plants and mice, is described above. It will be necessary to confirm the polymorphism of oxidoreductases and chelating proteins using electrophoretic maps, as well as conduct radiological and toxicological studies of both plant components and mouse biomaterials.

All animal experiments will be conducted in compliance with institutional and national ethical guidelines and approved by the relevant animal care and use committee.

The results of the research at the fourth stage will include instructions for pre-sowing and vegetative treatment of agricultural plants, the methodology of this treatment, an assessment of improving the quality of agricultural products and, as a result, the prospect of enabling more effective implementation of a preventive medicine program to improve the quality of nutrition and public health.

Overall, the proposed multi-stage research is expected to establish a novel agro technology that enhances food safety and public health by leveraging the natural defense mechanisms of plants through targeted application of flavonoids and other secondary metabolites.

Conclusion

The approach chosen by the authors proposes a paradigm shift from traditional strategies for reducing pollutants in soil and crops by modulating secondary metabolites such as flavonoids for safer and more cost-effective food production. The new agricultural technology will allow producing environmentally friendly crops on lands currently excluded from agricultural use due to pollution of the Russian Federation and Republic of Belarus and worldwide. A joint development of institutes of both countries has the potential to promote preventive medicine and sustainable agricultural technologies.

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