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ASSESSMENT OF THE ENVIRONMENTAL EFFECTS OF MILITARY ACTIVITIES
ON SOIL-PLANT SYSTEMS DURING THE WAR IN UKRAINE

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Abstract: A key area of research is the impact of various military-induced stresses on plants, with a particular emphasis on their productivity. This study assesses the impact of soils contaminated with radionuclides, heavy metals (Cd, Pb, Sr, Cs, etc.), and explosives (RDX, TNT, HMX, PENT, etc.) on specific physiological, genetic, biochemical, and growth parameters of plants. The rate of harmful substance transfer from soil to plants was assessed using several plant species. Based on the results obtained, promising species for soil restoration in war-affected areas were proposed and fundamental knowledge in the field of plant stress biology was obtained. The primary goal of this research is to investigate the molecular biological and genetic mechanisms of plant cell function using biotechnology, develop new technologies in cellular and genetic engineering, and study the adaptive response of plants to abiotic stress. The main scientific achievements include the investigation of the soil-plant system in a military exclusion zone containing explosive chemicals, as well as the study of the genetic hazard of radiation from radionuclide-contaminated environments and the ontogenesis of plants.

Keywords: contaminated environment, assessment, radionuclide, heavy metal, explosive chemicals.

1. Introduction

Researchers investigated several plants and phytoremediation methods for soils contaminated with radionuclides, heavy metals and explosives. Plant species common across various natural zones of Ukraine – such as willow, poplar, southern reed, spruce, and pine, are capable of accumulating radionuclides, explosive residues and products of their incomplete degradation. These products may serve as biological indicators of environmental pollution caused by military actions. At the same time, some of these species can be used for phytoremediation, for example, southern reed may be used for phytoextraction of trinitrotoluene from water bodies located in the combat zone. The study assesses the impact of soils contaminated with radionuclides, heavy metals (Cd, Pb, Sr, Cs, etc.), and explosives (RDX, TNT, HMX, PENT, etc.) on specific physiological, genetic, biochemical, and growth parameters of plants [1–4]. Transfer coefficients from soil to plant were estimated for several species. Based on the results, promising species for planting in war-affected areas will be proposed for soil restoration. In addition, the research will contribute to fundamental knowledge in plant stress biology [5].

The research team from ICBGE gained valuable experience in studying the effects of various military-induced stress factors on plants, with a particular focus on their productivity. The Department of Biophysics and Radiobiology (DBR), founded over 50 years ago, has been part of ICBGE since 1989. Currently, DBR research is devoted to the molecular and biological basis of

processes caused by chronic irradiation in the Chernobyl zone, as well as the important role of molecular recovery pathways in plant resistance to anthropogenic factors.

The main aim of the research is to study the molecular, biological, and genetic mechanisms of plant cell function using biotechnology methods; to develop novel technologies in cell and genetic engineering and to investigate the adaptive responses of plants to biotic and abiotic stresses. Therefore, assessing the impact of military actions during the war between Ukraine and Russia on the soil-plant system is essential for mitigating the consequences. Major research achievements of the Institute include: plant genetic transformation technologies; the production of recombinant proteins using plant systems; genetic behavior and plant lifespan within soil-plant systems in the Chernobyl alienated zone under the influence of chronic radiation from a nuclide-contaminated environment.

2. Materials and Methods

Biophysical and radiobiological approaches were applied to study the soil-plant system. Plant species were examined in several contaminated areas. Data collection and analysis of soil and plants from military-affected areas in northern and eastern Ukraine were carried out. Experimental soil plots located in military-affected sites were selected and characterized preliminarily based on contamination levels. Soil and plant samples were collected from these areas, and ex situ experiments were conducted to evaluate the effects of military-contaminated soils on plants. Estimation of bioremediation capacity of different plant species is important and relevant. The concentrations of pollutants and toxic substances in soil and plant tissue samples were determined using FTIR spectroscopy [6–9]. The comparative accumulation capacity of war-related contaminants in plant tissues was analyzed to assess their environmental fate under combat conditions.

For example, the objective of these studies was to determine damage responses in transgenic lines of *Arabidopsis thaliana* L. exposed to abiotic stress factors associated with military activities. Three transgenic lines of *Arabidopsis thaliana* L. were used in this experiment. The transgenic lines contain: *RPD3* – a histone deacetylase gene, *SIR2* – a gene encoding a histone deacetylase protein, *SU(VAR)* – suppressor variation gene and the reporter marker *GUS* – gene. The mentioned genes are involved in regulating structural changes in chromatin during mitosis. Interchromatid recombination between sister homological chromatid restored the interrupted version of the *GUS*-gene. This process in cells becomes apparent as a blue spot after addition to etiolated plant tissues X-Gluc (5-Bromo-4-chloro-indolyl- β -D-glucuronide Cyclohexylamine salt). The emergence of the blue spot as a result of the interchromatid exchanges marked by the *GUS*-gene in transgenic *Arabidopsis* lines due to the influence of different factors, which induce mitotic crossing-over in somatic cells [10].

PSII photosynthetic activity was evaluated using chlorophyll fluorescence induction curves recorded with a custom-built instrument. Data was obtained for 0.1 ms in the fast phase and 0.1 s in the slow phase of the induction curve. To estimate the efficiency of the photosynthetic apparatus, the parameters F_v/F_m (the maximum quantum yield of PSII) and the F_m/F_{st} (vitality index, characterizing integral activity) were calculated [11].

The MPM-100 was used to assess the effects of contaminated ambient on the relative contents of chlorophyll, anthocyanins, flavonols, and the nitrogen balance index in willow and poplar leaves [12].

To evaluate the genetic damage, *Tradescantia* clone 02 plants were grown over three consecutive flowering periods on soils with different levels of radionuclide contamination. Control soil samples were taken in the vicinity of Kyiv, whereas experimental soil samples were taken in

the vicinity of Chernobyl exclusion zone (30-km zone of the ChNPP) and the village of Kopachi (10-km zone of the ChNPP). The theoretical radiation dose accumulated after 1, 2 and 3 planting cycles during somatic reproduction of plants was calculated [13].

3. Results and discussions

As a result of missile strikes, structural damage to the roof of Unit 4 of the Chernobyl Nuclear Power Plant was observed (Figure 1).



Fig. 1. Structural damage to the roof of Unit 4 of the Chernobyl Nuclear Power Plant following a missile strike.

Although the fire beneath the new arch of shelter was extinguished, the possibility of additional radionuclide releases cannot be excluded. Radionuclide releases typically occur unevenly forming radionuclide spots. Dose assessment is complicated by the presence of solid radioactive fallout in the form of hot particles [14].

An increase in the second flowering peak of flax was observed compared to control plants. This may result in higher inflorescence sterility and decreased yield in the Chernobyl zone.

Proteomic analysis of 8th-generation mature soybean seeds grown in the Chernobyl zone and the first-year generation in uncontaminated soil was performed using two-dimensional gel electrophoresis. The analysis revealed 15 differentially abundant protein spots in the field samples and 11 in the clean soil comparison. These proteins were primarily associated with storage proteins, disease and defence responses and protein metabolism. The data suggests that hazardous heritable alterations accumulated during multigenerational growth under contaminated environmental conditions [15, 16].

Transgenerational adaptive plasticity does not appear to rely on low-frequency mutations. Our proteomic data suggest that epigenetic modifications may play a significant role in soybean, flax and *Arabidopsis thaliana* grown under chronic ionizing radiation.

Degenerative traits accumulated in seeds as a result of several generations of flax breeding. This process may gradually degrade both seed quality and the nutritional value of flax oil.

Prion-like proteins provide a unique mode of biochemical memory through self-guiding changes in protein folding, conformation and consequently function. Although intensively studied in fungi and mammals, they have not yet been discovered in plants. Our recent proteomic data reveal that cupin superfamily proteins contain a dis-folding structure [17].

Semi-sterile *Tradescantia* clone 02 plants were used as an effective biological indicator of mutagenic factors, particularly soil contamination with radionuclides and heavy metals. Plants were grown on control soils, soils from the 30-km zone, and soils from the 10-km zone of the Chernobyl Nuclear Power Plant. The specific activity of ^{241}Am in soils in the 30-km and 10-km zones was $87 \pm 7 \text{ kBq/m}^2$ and $13 \pm 2 \text{ kBq/m}^2$, respectively. The specific activity of ^{137}Cs in these soil samples was 20.8 and 1.12 kBq/kg. Trace amounts of ^{241}Am , and ^{137}Cs were detected in the control sample (1.1 kBq/kg). *Tradescantia* clone 02 plants were cultivated in these soils three times. Transgenerational accumulation and transmission of genetic damage were observed depending on the level of soil radionuclide contamination. Daughter plants may originate from both root systems and inflorescences of the parent plants (Table 1).

During a single vegetative growth cycle prior to flowering, the absorbed radiation dose from the soil was 0.04 cGy for control plants, 10 cGy for plants grown in soils from the 30-kilometer, and 0.52 cGy for plants cultivated in soils from the 10-km zone.

Flowers of *Tradescantia* clone 02 were examined in the morning using a light microscope. Pollen was stained with acetoorcein and examined at 500x magnification.

Table 1

Frequency of mutation events and morphological anomalies in *Tradescantia* clone 02 plants grown in soil contaminated with ^{241}Am and ^{137}Cs for three generations.

Soil	Number of generation	Dose, cGy	Frequency of the events	
			Mutation for 1000 stamen hairs	Morphisms, %
Control	1	0.04	0.3±0.2	2.2±2.0
Control	2	0.08	0.3±0.1	3.3±1.0
Control	3	0.12	0.2±0.1	2.0±1.0
10-km of the ChNPP	1	10.00	4.8±0.4	8.7±1.5
10-km of the ChNPP	2	20.01	8.4±0.3	12.2±1.2
10-km of the ChNPP	3	30.81	7.9±0.6	12.0±1.2
30-km of the ChNPP	1	0.52	3.0±0.8	6.8±2.1
30-km of the ChNPP	2	1.04	6.1±0.4	8.6±1.5
30-km of the ChNPP	3	1.55	4.5±0.4	8.0±1.6

Flowers involved in the formation of daughter shoots exhibited morphological anomalies. This observation suggests that mitotic crossing-over may occur prior to the emergence of daughter plants, which determines the morphological changes in the flowers. Radionuclide contamination of soil initiates unscheduled mitotic crossing-over in *Tradescantia* plants, which are a sterile (02) heterozygous clone carrying dominant blue and recessive pink alleles. Such events may lead to somatic segregation resembling the fertile parental lines *T. hirsutiflora* and *T. subcaulis*. An increased frequency of mitotic crossing-over represents one manifestation of genome destabilization. At the phenotypic level, the induced sister chromatid exchange observed in the applied plant test system may serve as a genetic marker of this phenomenon. The data presented in Table 1 show that the frequency of recessive mutations in plants grown in contaminated soil is higher, as is the frequency of morphoses in plants. However, the results indicate that plants adapt to the increased level of soil contamination, so it is inappropriate to consider dose dependence at low doses in this case. Growing *Tradescantia* in radionuclide-contaminated soils leads to unscheduled mitotic crossing-over in somatic cells, which leads to an increase in the frequency of recessive mutations.

Conventionally, radiation-induced cellular damage is classified as reversible or irreversible. Irreversible damage includes cell death, apoptosis and necrosis. Reversible damage encompasses morphological, physiological and biochemical alterations in surviving cells. These can involve radiation-reversible modifications of the plasma membrane and organelles, including changes in membrane-bound calcium channel proteins, membrane domains, micromorphology of cell surfaces, intercellular contacts, cytoskeleton, and changes in the structure of mitochondria and chloroplasts, endoplasmic reticulum, Golgi apparatus, and lysosomes. In addition, reversible damage includes chromosomal aberrations and micronuclei, as a consequence of the latter, nuclear protrusion, and changes in DNA packaging. The enhancers of radiation damage include not only those cellular metabolic processes that ultimately lead to increased damage, but, in our opinion, there are also special mechanisms of the cell's response to various stress factors. Such cellular mechanisms include crossing-over, synthesis of chaperones, folding proteins and the presence of viroids in the cell. Cellular responses to different types of radiation are not strictly specific, and can be considered as a response to general stress, similar to that observed after exposure to various damaging factors on the cell.

Additional methods, such as histochemical detection of proteins (e.g., *GUS* gene products), identification of specific cellular organelles and cytoskeleton markers, analysis of alterations on the cell surface and in different membrane domains, application of carrier compounds, and other cellular or molecular biological assays, may be incorporated into generally accepted protocols for the assessing stress-induced cellular changes.

Proteins such as prions can remain in the cell for a long time and cause long-term irreversible damage to the cellular protein structure. That is, an infectious protein arises from a normal cellular protein through modification. Cellular prions differ from infectious prions only in their tertiary structure. It is known that in the infectious protein, the prion PrP^{Sc}, many more so-called β -sheets were found - about 40% (while in the non-infectious cellular protein PrP^C - about 3%) - and a somewhat smaller number (43 - 34%) of so-called α -helices [18]. Moreover, the infectious protein, which forms amyloid strands, is more resistant to proteolysis compared to its cellular homologue PrP^C. Based on this observation, it may be assumed that proteins normally present in the cell, under the influence of radiation, could change the configuration of the tertiary structure from α - to β -structure. Such structural alterations may be stable and lead to significant morphological consequences, resembling prion-like mechanisms.

Thus, the data presented above suggest that somatic cell genome instability induced by radionuclides represents different stages in the transfer and realization of ionizing radiation energy within the cell. Radiation-induced energy transfer contributes not only to primary structural damage of cellular components but also to disturbances in energy balance of protein synthesis, proteolysis, and folding. These are associated with various biological mechanisms that sustain the damaging effect of radiation.

The explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), a major component of munitions, is used extensively in military operations and training ranges, resulting in widespread RDX contamination in groundwater and aquifers. RDX is toxic; however, its removal from training ranges is logistically challenging, with no cost-effective and sustainable solutions available [2]. Using transgenic technologies, the above-mentioned authors engineered *Thalassia cress* (*Arabidopsis thaliana*) and switchgrass (*Panicum virgatum*) to express two genes, *xplA* and *xplB*, encoding RDX-degrading enzymes from the soil bacterium *Rhodococcus rhodochrous* 11Y, enabling the breakdown of this xenobiotic in laboratory studies. A three-year field trial demonstrated that XplA/XplB switchgrass has in situ efficacy, with potential application for detoxifying RDX on live-fire training ranges, munitions dumps, and minefields.

4. Conclusion

The consequences of military activities may be mitigated through various approaches, from political-economic actions to phytoremediation of the soil, but the genetic consequences are transmitted through inheritance for many years [19, 20, 21]. After a high-explosive bomb hit the roof of the sarcophagus of Unit 4 of the Chernobyl nuclear reactor, a fire broke out inside, releasing additional radionuclide and toxic chemical substances into the environment. These contaminants are distributed in the vicinity of the shelter in a localized and uneven manner. Therefore, quantifying anthropogenic substrates in the soil presents significant challenges [22].

The study highlights the importance of investigating soil-plant systems within a military exclusion zones contaminated by explosive chemicals, as well as the study of the genetic hazard of radiation from radionuclide-contaminated environments and the ontogenesis of plants.

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ОЦЕНКА ЭКОЛОГИЧЕСКОГО ВОЗДЕЙСТВИЯ ВОЕННЫХ ДЕЙСТВИЙ ВО ВРЕМЯ ВОЙНЫ НА УКРАИНЕ НА ПОЧВЕННО-РАСТИТЕЛЬНЫЕ СИСТЕМЫ

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Резюме: Ключевым направлением исследований является изучение влияния различных стрессов, связанных с военными действиями, на растения, с особым акцентом на их продуктивность. Оценка влияния почв, загрязненных радионуклидами, тяжелыми металлами (Cd, Pb, Sr, Cs и др.), и взрывчатыми веществами (RDX, TNT, RDX, HMX, PENT и др.), на некоторые физиологические, генетические, биохимические и ростовые реакции растений. Скорость перехода вредных веществ в систему почва-растение будет оценена с использованием нескольких видов растений. На основании полученных результатов будут предложены перспективные виды для посадки в зонах военных действий с целью восстановления почв, а также будут получены фундаментальные знания в области биологии стресса растений. Основной целью данного исследования является изучение молекулярно-биологических и генетических механизмов функционирования растительных клеток с использованием биотехнологии, разработка новых технологий в области клеточной и геномной инженерии, а также изучение адаптивного ответа растений на абиотический стресс. К основным научным достижениям относятся исследование системы «почва-растение» в военной зоне отчуждения, содержащей взрывоопасные химические вещества, а также влияние радиации от загрязненных радионуклидами сред на оценку генетической опасности и продолжительность жизни растений.

Ключевые слова: загрязненная окружающая среда, оценка, радионуклид, тяжелый металл, взрывчатые химические вещества.

UKRAYNADA MÜHARIBƏ DÖVRÜNDƏ HƏRBI FƏALİYYƏTİN TORPAQ-BİTKİ SİSTEMLƏRİNƏ ƏTRAF MÜHİTƏ TƏSİRİNİN QIYMƏTLƏNDİRİLMƏSİ

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Xülasə: Əsas tədqiqat sahəsi müxtəlif hərbi mənşəli stresslərin bitkilərə təsiridir və onların məhsuldarlığına xüsusi diqqət yetirilir. Bu tədqiqat radionuklidlər, ağır metallar (Cd, Pb, Sr, Cs və s.) və partlayıcı maddələr (RDX, TNT, RDX, HMX, PENT və s.) ilə çirklənmiş torpaqların bitkilərin müəyyən fizioloji, genetik, biokimyəvi və böyümə reaksiyalarına təsirini qiymətləndirir. Zərərli maddələrin torpaq-bitki sisteminə ötürülmə sürəti bəzi bitki növündən istifadə etməklə qiymətləndirilir. Əldə edilən nəticələrə əsasən, torpaq bərpa üçün müharibə zonalarında əkin üçün perspektivli bitkilər təklif ediləcək və bitki stress biologiyası sahəsində fundamental biliklər əldə ediləcək. Bu tədqiqatın əsas məqsədi biotexnologiyadan istifadə edərək bitki hüceyrə funksiyasının molekulyar bioloji və genetik mexanizmlərini araşdırmaq, hüceyrə və gen mühəndisliyində yeni texnologiyalar hazırlamaq və bitkilərin abiotik stressə adaptiv reaksiyasını öyrənməkdir. Əsas elmi nailiyyətlərə partlayıcı kimyəvi maddələr olan hərbi təcrid zonasında torpaq-bitki sisteminin öyrənilməsi, eləcə də radionuklidlə çirklənmiş mühitlərdən şüalanmanın genetik təhlükəsi və bitkilərin ontogenezinin araşdırılması daxildir.

Açar sözlər: çirklənmiş mühit, qiymətləndirmə, radionuklid, ağır metal, partlayıcı kimyəvi maddələr.