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Research Article

# Epigenetic factors of the effect of UV-C and X-ray presowing seeds radiation exposure in *Matricaria chamomilla* L. genotypes

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Secondary metabolites,
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**Abstract:** In a series of experiments using both X-ray and UV-C radiation exposure a parallel study of several pharmacological characteristics of the *Matricaria chamomilla* L. genotype group was carried out. The data concerning the changes in the productivity of pharmacological raw materials and stimulation of the synthesis of low molecular weight antioxidants as markers of secondary metabolism induction have been published earlier. In this study, the data on the relationship between the stimulation of the synthesis of secondary metabolites under different types of irradiation and the epigenetic changes in the plant organism are presented. It was shown that DNA methylation was switched to the *de novo* mode in plants of all studied genotypes of *M. chamomilla* under both types of irradiation. That indicates changes in the epigenetic program of the plant organism. Comparison of the epigenetic pattern between control and irradiated samples, based on the difference in DNA methylation patterns in terms of a statistical indicator, shows that there is no unambiguous relationship between the epigenetic changes and increasing yield of antioxidant synthesis. This is additional evidence of the diversity of metabolic rearrangements and adaptive strategies of the plant organism under radiation exposure even within one species.

## 1. INTRODUCTION

One of the directions of modern pharmacology is the identification of substances that are effective for medical practice and the stimulation of their formation in plant materials. The study of plant responses to stress factors has shown the possibility of stimulating the synthesis of secondary metabolic products, which include substances with antioxidant, anticancer, immunomodulatory, and anti-inflammatory effects (Dai & Mumper, 2010; Hassan *et al.*, 2017; Jan *et al.*, 2012; Kaur & Mondal, 2014; Klein *et al.*, 2018). The most effective approach is

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associated with the use of both ionizing and UV radiation with different wavelengths (Alothman *et al*., 2009; Hassan *et al*., 2017; Jan *et al*., 2012; Klein *et al*., 2018; Nocchi *et al*., 2020).

Presowing irradiation of medicinal plants has several biotechnological advantages. It is based on the systemic and long-term response of the organism to a single exposure. Presowing irradiation leads to remote effects, long-term changes in metabolism, and stimulation of the production of target substances in newly formed non-irradiated plant organs, for example, inflorescences during weeks or months after the exposure. It is crucial to utilize exposure in "low" doses that promote the production of target substances without diminishing the yield of pharmacological raw materials. Furthermore, such exposure can even stimulate it in certain species and varieties of plants (Sokolova *et al*., 2021).

A parallel study investigating various pharmacological characteristics of the *Matricaria chamomilla* L. genotype group was conducted through a series of experiments utilizing both X-ray and UV-C radiation exposure. The data concerning the changes in the productivity of pharmacological raw materials and stimulation of the synthesis of low molecular weight antioxidants as markers of secondary metabolism induction were published earlier.

Significant differences were observed in the stimulation of medicinal raw material (inflorescences) yield among different chamomile genotypes exposed to X-ray and UV-C irradiation (Sokolova *et al*., 2021). Also there was indicated a difference in the intensity of stimulation of the specific content of marker metabolites-antioxidants. This phenomenon suggests the involvement of various biophysical and biochemical mechanisms that influence sensitivity to specific types of radiation, along with potential variations in the development of protective reactions (Zhuk *et al*., 2021).

Recent-year research has significantly changed our understanding of the formation of the metabolic organism response to radiation. Switching of the activity of dozens and even hundreds of genes in the first minutes after the radiation exposure was established (Coleman *et al*., 2005). Significant rearrangements of the methylome under different modes of irradiation (Sokolova *et al*., 2013; Kravets *et al*., 2013, Kravets & Sokolova, 2020), large-scale changes in the proteome, stimulation of not only reparative and protective mechanisms, but also restructuring of the entire metabolon including various blocks of both primary and secondary metabolism (Danchenko *et al*., 2009; Klubicova *et al*., 2013) became known with the use of "omic" technologies in a radiobiological experiment. These facts not only have made a significant contribution to the transformation of the radiobiological paradigm, but are also important from a practical point of view.

The question arises as to the extent to which global metabolic changes in response to stress are linked to the release of practically valuable substances. Hence, one of the objectives of the research was to evaluate the correlation between the extent of generalized metabolic switching during pre-sowing irradiation of medicinal plant seeds and the yield of target metabolites. One possible approach to addressing this issue was to assess the overall epigenetic differences between control and irradiated samples, followed by comparison with antioxidant yield.

Currently, research on epigenetic mechanisms, such as gene expression mechanisms, is being conducted in various directions: from studying changes in chromatin organization to exploring mRNA translation and interference mechanisms under the influence of climatic factors and various stresses (Flores *et al*., 2013; Ng & Bird, 1999; Teif, 2015). DNA methylation stands out as the most studied chromatin modification, serving as a key factor in gene expression control and the primary mechanism of transgenerational "epigenetic memory" (Hauser *et al*., 2011). This process constitutes a crucial, inseparable component of the multilevel epigenetic regulation system (Flores *et al*., 2013; Xu *et al*., 2019). Consequently, in addressing several practical issues, the "distance" between methylation patterns is utilized as a measure of the impact on epigenomes by environmental or stress factors (Flores *et al*., 2013).

The aim of this research was to investigate changes in the methylome under radiation exposure of different physical natures and to evaluate the relationship between these changes and the yield of secondary metabolites. In addition to a visual analysis of electrophoregrams, valuable information was derived from the assessment of their quantitative characteristics, illustrating differences in the distribution of ITS-ISSR-PCR amplicons as restriction products in control and exposed variants.

## 2. MATERIAL and METHODS

Research was carried out on 8 genotypes of chamomile: 1 - generative breed of the mutant Perlyna Lisostepu (Ukraine); 2 - Quedlinburg variety (Germany); 3 - Goral variety (Slovenia); 4 - variety Azulena (Russia); 5 - Zlaty Lan variety (Poland); 6 - Perlyna Lisostepu variety (Ukraine). Additionally, unsorted material, specifically the edaphic ecotypes, were included in the study: 7- from the Golden Garden supplier (Ukraine), hereinafter referred to as the Golden Garden ecotype; 8 – from the supplier Seed Era (Ukraine), hereinafter referred to as the Seed Era ecotype. The varietal material was obtained from the Central Research Station of Medicinal Plants of the Institute of Agroecology and Nature Management of the National Academy of Sciences of Ukraine in Lubny. The experiment was repeated three times. Dry seeds were exposed using the RUM-17 X-ray installation (Russia) at a dose of 10 Gy, with a dose rate of 1.42 cGy/s. The choice of the X-ray irradiation dose was based on the authors' results, confirmed by a patent (Shilina *et al*., 2018). UV-C exposure was conducted at a dose of 10 kJ/m² using an OBM-150 M installation (Ukraine) with two Philips Special TUV 30 W lamps (Netherlands). The choice of the UV-C irradiation dose was based on preliminary studies, also confirmed by a patent (Kravets *et al*., 2021). DNA methylation research was carried out through restriction analysis followed by ISSR-ITS-PCR (Ausubel, 2004; Hernández *et al*., 2013).

DNA was isolated from the plant vegetative mass during the flowering phase using a set of reagents ZymoResearch (Quick-DNA Plant/Seed Miniprep Kit) according to the manufacturer's protocol. The nativeness of the isolated DNA was checked in a 1.7% agarose gel with TBE buffer in the presence of ethidium bromide and visualized on a UV transilluminator (Figure 1) When setting up electrophoresis 5 µL of DNA solution was put into the "pocket" of the gel. GeneRuler 50 bp was used as a molecular weight marker. Three types of markers were used for PCR: ISSR-5 (CAC-ACA-CAC-ACA-CAC-AAC), ITS (ITS1 (TCC-GTA-GGT-GAA-CCT-GCG-G) and ITS4 (TCC-TCC-GCT-TAT-TGA-TAT-GC) and ready-to-use PCR MIX 2x-R («Neogene», Ukraine).

ISSR–PCR reaction mix 25 µL volume included: 12.5 µL PCR MIX 2x-R), 1.75 µL ISSR/OPA09 markers, 5.75 µL deionised water and 5 µL genome DNA. ITS-PCR reaction mix 25 µL volume included: 12.5 µL PCR MIX 2x-R), 0.6 µL ITS1 marker, 0.6 µL ITS4 marker, 5.75 µL deionised water and 5 µL genome DNA. Amplification with ISSR-primers included stages: initial denaturation 4 min under 94 ºC, 40 cycles; denaturation under 94 ºC – 45 sec., annealing under 52 ºC – 45 sec., elongation under 72 ºC – 45 sec.; final elongation 7 min under 72 ºC. Amplification with OPA09-primers included stages: initial denaturation 5 min under 94 ºC, 40 cycles; denaturation under 94 ºC – 40 sec., annealing under 36 ºC – 40 sec., elongation under 72 ºC – 2 min.; final elongation 10 min under 72 ºC. Amplification with ITS-primers included stages: final denaturation 5 min under 94 ºC, 40 cycles; denaturation under 94 ºC – 40 sec., annealing under 52 ºC – 40 sec., elongation under 72 ºC – 2 min; final elongation 10 min under 72 ºC.

For restriction analysis two types of restrictase-isoschizomers were used: MspI (C…C*GG and C…CGG) and HpaII (C…CGG), (Fermentas, Litva). Enzymes HpaII and MspI cleave the CCGG sequence, but the action of HpaII is directed only at unmethylated cytosine. MspI, as an isoschizomer of HpaII, cleaves both methylated and unmethylated sites. Restriction reaction with endonuclease MspI was in volume 20 µL, included 0.6 u of enzyme (0.6 µL), 6 µL

10xBuffer Tango, 500 ng genome DNA (5 µL), and 5.75 µL deionised water. Reaction mix for the restriction with HpaII in volume 20 µL included 0.2 u of enzyme (0.2 µL), 6 µL 10xBuffer Tango, 500 ng genome DNA (5 µL), 8.8 µL of deionised water. The reaction was done 3 h under 37 ºC, termination of the reaction – 20 min under 65 ºC (for HpaII) and 20 min under 80 ºC (for MspI).

The products of PCR and restriction were separated in 1.7% agarose gel with TBE buffer in the presence of ethidium bromide and visualized on UV transilluminator. When setting up electrophoresis the same volume of PCR and restriction products (5 µL) was add into the gel "pocket". GeneRuler 50 bp was used as a molecular weight marker.

As an indicator of the difference between the set of electrophoregrams of control and exposed samples the indicator D = epigenetic distance (hereinafter - ED) was used. It was calculated similarly to the estimation of the genetic distance according to Nei (Nei, 1974).

At the same time D = 0 with absolute coincidence between the set of bands in the electrophoretic spectra that are compared; D = 1 under conditions of complete difference between the set of amplicons in the electrophoretic spectra.

## 3. FINDINGS

Assessment of the isolated DNA's quality confirms its high purity and integrity (Figure 1) in both control and exposed experimental variants, enabling further restriction analysis. Analysis of the data obtained revealed that both ionizing and UV-C pre-sowing irradiation of seeds led to a transition in the DNA methylation process from maintenance to de novo mode across all genotypes during plant formation (Figure 2 and 3).

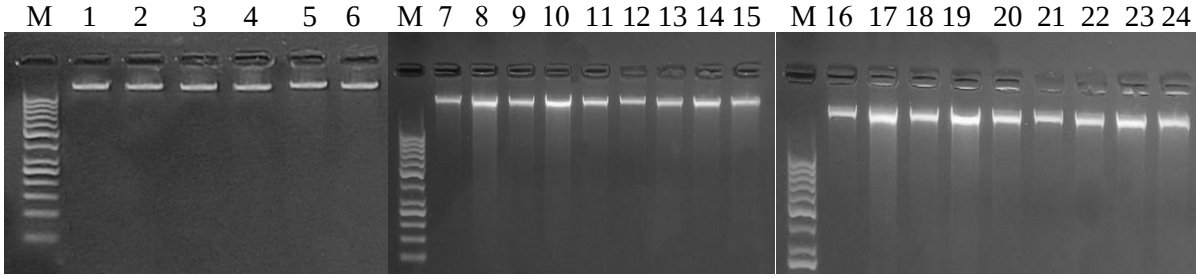


**Figure 1.** Electrophoregram of the isolated DNA nativity.
M – molecular weight marker GeneRuler 50 bp, 1 – genotype 1, control; 2 – genotype 1 + UV-C; 3 – genotype 1+ x-ray; 4 – genotype 2, control; 5 – genotype 2+ UV-C; 6 – genotype 2+ x-ray; 7 – genotype 3, control; 8 – genotype 3+ UV-C; 9 – genotype 3+ x-ray; 10 – genotype 4+ control; 11 – genotype 4+ UV-C; 12 – genotype 4+ x-ray; 13 – genotype 5, control; 14 – genotype 5+ UV-C; 15 – genotype 5+ x-ray; 16 – genotype 6, control; 17 – genotype 6+ UV-C; 18 – genotype 6+ x-ray; 19 – genotype 7, control, 20 – genotype 7+ UV-C, 21 – genotype 7+ X-ray; 22 – genotype 8, control; 23 – genotype 8, UV-C; 24 – genotype 8 + x-ray

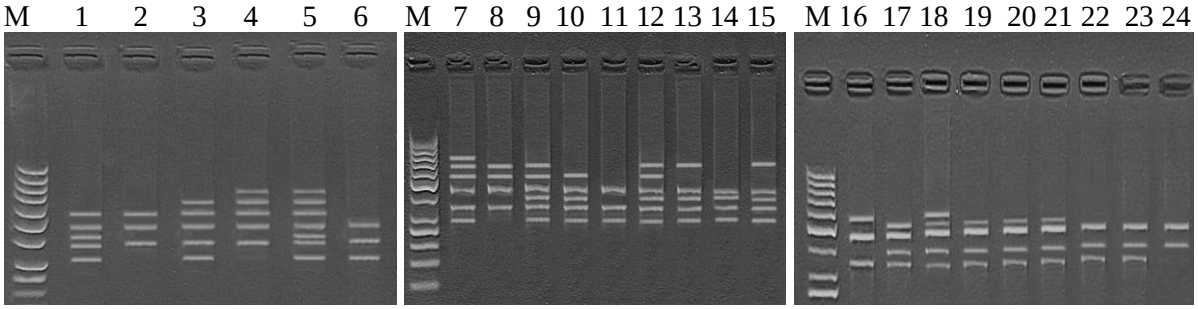


**Figure 2.** Amplification of HpaII restricts with ISSR markers (Designation as in Figure 1).

Significant changes in methylation of HpaII restriction sites were observed for minisatellite sequences (Figure 2) across all genotypes. In the mutant Perlyna Lisostepu variety, methylation patterns (Figure 2, positions 1-3) were altered relative to the control variant under both UV-C and X-ray exposure. Notably, relatively large amplicons of 600, 500, and 400 bp were maintained for all variants. However, amplicons of 450 and 300 bp disappeared under UV-C exposure, indicating the removal of methylation in specific areas of the minisatellite sequence. Upon exposure to X-rays, a "short" amplicon of 300 bp was retained, while a relatively "long" one of 700 bp appeared, also suggesting the removal of methylation in certain areas of the minisatellite sequence, given that HpaII restrictase cleaves sites with unmethylated cytosine (C…CGG).

Similarly, changes in methylation of HpaII restriction sites were observed for minisatellite sequences in the Quedlinburg variety (Figure 1, positions 4-6). Amplicons of 500 and 400 bp were retained for both control and exposed variants. Under UV-C exposure, "long" amplicons of 600-800 bp were maintained, and "short" ones of 300 and 450 bp appeared. Additionally, amplicon 300 bp appeared under X-ray exposure, although with this type of irradiation, all "long" amplicons disappeared.

For the Goral variety (Figure 2, positions 7-9), changes in HpaII restriction site methylation within minisatellite sequences under UV-C and X-ray irradiation were also noted. Across all variants, amplicons of 700, 600, 500, and 350 bp were maintained.

The most significant difference was observed for the Azulena variety under UV-C exposed variants (Figure 2, positions 10-12). Amplicons of 500, 350, and 300 bp were present for both control and exposed variants. Under UV-C exposure, the longest band of 600 bp disappeared, reflecting an increase in methylation yield in the satellite DNA sequence. Conversely, under X-ray exposure, a “long” amplicon of 700 bp appeared, indicating the removal of methylation in certain areas of the minisatellite sequences.

Similarly, Zlaty Lan variety plants reacted to irradiation, with the largest change in methylome observed under UV-C exposure. Across all variants, only amplicons of 500, 400, 350, and 300 bp were retained (Figure 2, positions 13-15). Perlyna Lisostepu variety was characterized by the appearance of additional amplicons of 550 and 400 bp under both types of exposure (Figure 2, positions 16-18) (Figure 2, positions 16-18).

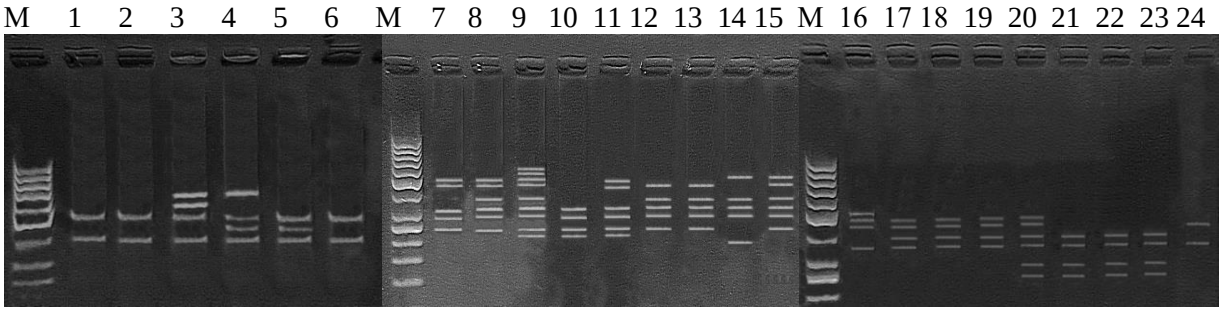


**Figure 3.** Amplification of MspI restricts with ISSR markers (Designation as in Figure 1).

There were no changes observed in the methylome of the Golden Garden variety under both types of radiation exposure (Figure 2, positions 19-21). The Seeds Era variety demonstrated the same set of amplicons under UV-C exposure as the control, but under X-ray exposure, the 350 bp amplicon disappeared (Figure 2, positions 22-24). Amplification of HpaII restricts with ITS markers did not indicate any difference between the amplicon sets in both control and exposed variants.

Significant changes in methylation of MspI restriction sites were also observed within minisatellite sequences (Figure 3). For example, in the mutant Perlyna Lisostepu variety, amplicons of 550 and 400 bp were maintained for all variants, and two "long" amplicons of 800 and 700 bp appeared additionally after X-ray exposure (Figure 3, positions 1-3). Similarly, for the Quedlinburg variety, amplicons of 550 and 400 bp were maintained for all variants. However, the "long" amplicon of 800 bp disappeared under both types of exposure, and the 450 bp amplicon disappeared only under X-ray irradiation (Figure 3, positions 4-6).

Significant rearrangements of the methylome were observed for the Goral variety, where amplicons of 550, 500, 350, 300, and 250 bp were detected for all variants (Figure 3, positions 7-9).

For the Azulena variety, the most significant changes (Figure 2, positions 10-12) relative to the control were observed under UV-C irradiation. Amplicons of 500, 350, and 300 bp were presented in both control and irradiated variants. Under UV-C exposure, the longest amplicon of 600 bp disappeared, indicating an increasing methylation level for this satellite sequence. Conversely, under X-ray exposure, a long amplicon of 700 bp appeared, indicating the removal of methylation in certain areas of the minisatellite sequences.

For the Zlaty Lan variety, general amplicons of 400, 350, and 300 bp were observed for both control and exposed variants. Under UV-C exposure, a new band of 550 bp appeared (Figure 3, positions 13-15). The 600 bp amplicon appeared only in the control variant of the Perlyna Lisostepu variety and disappeared under both types of irradiation. Moreover, bands of 550, 500, and 350 bp were common for all variety variants (Figure 3, positions 16-18).

For the Golden Garden ecotype, amplicons of 400 and 350 bp were common for all variants of the experiment. Under UV-C irradiation, amplicons of 550, 500, 400, and 350 bp were maintained, and amplicons of 300 and 250 bp appeared in the control variant. The same amplicons also appeared under X-ray irradiation. Longer amplicons presented in control and UV-C exposed variants disappeared (Figure 3, positions 19-21).

The 350 bp amplicon was common for all variants of the Seeds Era variety. Under UV-C exposure, there were no changes within the set of 4 amplicons relative to the control variant, but under X-ray exposure, the additional 500 bp amplicon appeared (Figure 3, positions 21-24).

Thus, no changes in the methylation pattern of transcribed DNA sequences were detected for most Perlyna Lisostepu genotypes. The appearance of a new 600 bp amplicon during amplification of MspI restricts with ITS markers was detected only under UV-C exposure of the Perlyna Lisostepu variety.

At the same time, changes in DNA methylation patterns, mostly through satellite DNA sequences, have been significant for gene expression regulation. Depending on current data, satellite DNA is considered to play an important role in chromatin remodeling, altering its effect on denaturation, and increasing the availability of transcription and repair factors. Thus, it could immediately affect DNA sensitivity to radiation damage and determine epigenetic control of metabolic processes (Flores *et al*., 2013; Kravets & Sokolova, 2020; Ng & Bird, 1999; Teif, 2015; Xu *et al*., 2019).

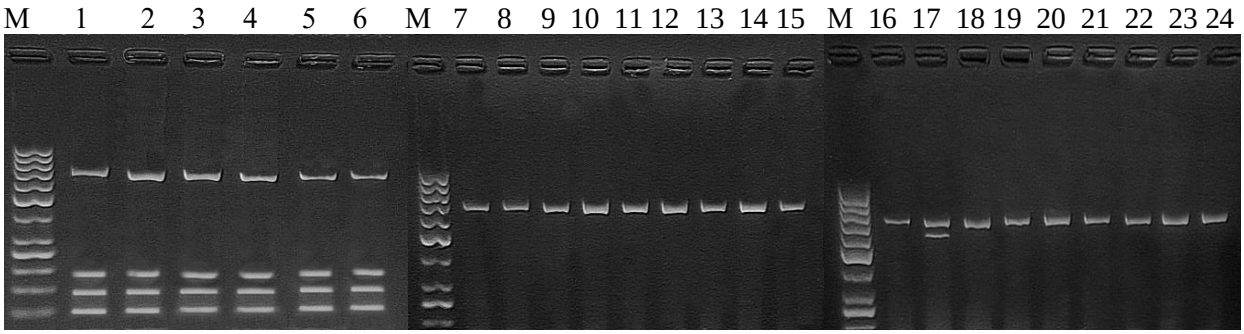


**Figure 4.** Amplification of MspI restricts with ITS markers (Designation as in Figure 1).

The restriction analysis with restrictases - isoschizomers did not indicate any general trend of changes in the overall DNA methylation yield under UV-C or X-ray exposure across the studied genotypes, which could have been reflected in a common increase or decrease in the yield of "long" amplicons. However, significant changes in the DNA methylation pattern were observed. These results confirm our previous findings and those of other studies, indicating that phenotypic variation is not attributed to the overall DNA methylation yield but rather to its pattern (Xu *et al*., 2019; Kravets & Sokolova, 2019; Kravets & Sokolova, 2020).

## 4. DISCUSSION and CONCLUSION

The comparison of the epigenetic distance between control and exposed variants with the experimental results of pharmaceutical raw material yield and antioxidant content is presented in Table 1 (Sokolova *et al*., 2021; Zhuk *et al*., 2021). The highest deviation rates from the control methylation pattern under both types of exposure were observed for the mutant of the Perlyna Lisostepu variety. Additionally, this genotype exhibited greater responsiveness to UV-C irradiation in terms of pharmaceutical productivity.

**Table 1.** Epigenetic distance of exposed variants relative to the control.

| Variety | D-epigenetic distance of exposed variants relative to the control | | Variety | D- epigenetic distance of exposed variants relative to the control | |
|---|---|---|---|---|---|
| | 10 Gy | 10 kJ/m$^2$ | | 10 Gy | 10 kJ/m$^2$ |
| Mutant of Perlyna Lisostepu variety | 0.083 | 0.04 | Zlaty Lan | 0.01 | 0.01 |
| Quedlinburg | 0.01 | 0.04 | Perlyna Lisostepu | 0.04 | 0.04 |
| Goral | 0.02 | 0.03 | Golden Garden | 0.02 | 0.01 |
| Azulena | 0.04 | 0.01 | Seed Era | 0 | 0.03 |

For the Quedlinburg variety, a higher distance between the methylation patterns of the control and exposed variants was observed under UV-C exposure. The yield of inflorescences was higher under X-ray exposure, and an increase in the specific yield of flavonoids was observed under both types of irradiation.

In the case of the Azulena variety, there is a correlation between a high rate of DNA methylation pattern deviation under X-ray exposure relative to the control (0.04) and an increase in the yield of both inflorescences and flavonoids. The Perlyna Lisostepu variety exhibited the highest specific content of flavonoids in the control variant. A significant increase in the indicator and the yield of inflorescences was observed under both types of exposure, accompanied by similar deviations in the DNA methylation pattern between exposed variants and the control.

The comparison of the degree of deviation in the methylation pattern with indicators of pharmaceutical productivity indicates the absence of a straightforward relationship between

these quantitative characteristics. This result suggests a diversity even within one species. In the context of biotechnological implementation of various types of irradiation, this diversity should be considered as a basis for exploring a wider range of useful substances that could be produced by plants under stress exposure.

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## Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

## Authorship Contribution Statement

**Daryna Sokolova**: Investigation, Methodology, Visualization, Software, Writing original draft. **Vladyslav Zhuk**: Investigation, Methodology, Visualization, Software. **Ludmila Hlushchenko**: Resources. **Alexandra Kravets**: Investigation, Methodology, Formal Analysis, Validation and Writing original draft, supervisor.

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