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Short Note

Study on the ultraviolet radiation impact on agro-industrial plant – preliminary results of cytogenetic and biochemical investigations**

Mihaela Hoștină[1], *Lăcrămioara Oprică*[2], *Gabriela Vochița*[3], *Mihaela Răcuciu*[4]*, *Dorina Creangă*[1]

[1]Department of Physics, Alexandru Ioan Cuza University, 11 Bld. Carol I, 700506, Iasi, Romania
[2]Department of Biology, Alexandru Ioan Cuza University, 11 Bld. Carol I, 700506, Iasi, Romania
[3]Department of Genetics, Institute of Biological Research Iasi, 47 Lascar Catargi Street, 700107, branch of NIRDBS Bucharest, Romania
[4]Department of Environmental Sciences and Physics, Faculty of Sciences, Lucian Blaga University of Sibiu, 5-7 Dr. I. Ratiu St., 550012, Sibiu, Romania

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A b s t r a c t. Although we are currently facing the danger of ultraviolet radiation from solar emissions due to the weakening of ozone layer protection, their interaction with vegetation could have useful applications. We performed experiments on a widely cultivated plant species, *Cucurbita pepo*, by applying controlled UV-C radiation exposure to freshly germinated seeds. Microscopic images were captured using a high-resolution video camera on root tissue samples prepared according to a well-established protocol. Biochemical analyses were focused on the representative antioxidant enzymes, superoxide dismutase and catalase, as well as on photosynthetic pigments. The cytogenetic investigations revealed various chromosomal changes, mainly chromosome bridges, expelled and lagging chromosomes and C-metaphases, as well as combinations thereof. The biochemical analyses revealed mainly reduced superoxide dismutase activity but greatly increased catalase activity, together with similar, although less extensive, variations in malondialdehyde content and progressive positive variations in soluble proteins. Stimulated photosynthetic efficiency was also found for some seedling samples, based on the ratio of chlorophyll concentrations, which seems to be consistent with the statistical results on the stem length of seven-day-old seedlings. Thus, studying controlled UV-C irradiation of seeds could reveal possible applications in the mutagenesis of plants of agro-industrial interest.

K e y w o r d s: catalase, superoxide dismutase, chromosomal aberrations, photosynthesis efficiency, photolysis of water

1. INTRODUCTION

High-energy ultraviolet rays, such as those in the UV-C range, can have a notable impact on the biosphere, even if they are random in nature, due to their ability to damage or modify biomolecules such as DNA, with consequent genetic changes in spontaneous or cultivated vegetation. An ambient level of total UV radiation (290-385 nm) has been estimated at approximately 12.23 W m^{-2} (Sahan, 2019). Some laboratory experiments have shown that moderate exposure to UV radiation can also induce genetic changes but with potentially beneficial effects in plant organisms, increasing crop yield and improving resistance to environmental stress. Thus, biotechnological approaches involving plant irradiation may include, in addition to seed sterilization, the induction of genetic mutations, based on the ability of radiation to damage or modify biomolecules such as DNA, with consequent chromosomal changes.

Exposure of plant seeds (anis, fennel, cumin) to UV-C radiation (254 nm, 105 W m^{-2}, up to 45 min) has been shown to increase antioxidant enzyme activity over a relatively short exposure period, while a longer exposure period induced opposite effects (Kamel *et al.,* 2022). Ebrahim *et al.* (2022) highlighted certain modifications in the content of phenols and flavones in *Salvia hispanica* L. seeds exposed to ultraviolet light (for 1, 2, 3, and 4 h at 5 cm and

*Corresponding author e-mail: mihaela.racuciu@ulbsibiu.ro
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20 cm distances from the ultraviolet source) in the form of positive variations for relatively low energy exposure and negative variations for relatively intense irradiation. The results reported by Neelamegam and Sutha (2015) for peanut plants showed that UV-C irradiation for up to 1 h increased seedling vigor and biomass, without significant adverse effects on seed germination, seedling growth, and peanut productivity. Other authors (Sadeghianfar *et al.,* 2019) have obtained stimulation of germination of corn and sugar beet seeds after exposure to UV-C radiation (254 nm, up to 12 h), and roots and stems increased significantly in seedlings grown from irradiated seeds. Nakamura *et al.* (2021) have reported a remarkable increase in chromosomal changes in transgenic *Arabidopsi*s plants after exposure to UV-C radiation.

Çavuşoğlu *et al*. (2022) found chromosomal changes in *Allium cepa* roots after exposure to UV-C (6 W lamp at 20 cm above the samples) for 72 h.

Bajaj *et al.* (2023) reconsidered these experiments with reduced UV-C irradiation of *Allium cepa* bulbs for several hours (1-2-4 h) and also identified various types of chromosomal aberrations like vagrant, laggard, and sticky chromosomes, C-mitoses, chromosomal bridges, multipolar anaphases, and others. Hammok and Esho (2022) found reduced chlorophyll content in adult pumpkin plants developed from seeds exposed to UV-C for tens of minutes.

According to studies carried out on corn seeds, different times of exposure to UV-C induced either stimulation or reduction of biological parameters of plants **(**Garbeles *et al.,* 2024). Measurements on 10-day-old seedlings showed that irradiation for up to one hour resulted in an increase in stem and root length, while longer irradiation times (such as two hours) did not produce significant changes. UV-C irradiation for up to 1.5 h stimulated wheat seed germination, as reported by Rupiasih and Vidyasagar (2016), and the chlorophyll A/B ratio remained unchanged, while the roots and shoots of seedlings were reduced compared to non-irradiated control plants. Tripathi *et al.* (2024) reported that UV-C irradiation of mung bean seeds improved the content of bioactive components in the sprouts, which promises health benefits for consumers.

The pumpkin we chose to study is used in human nutrition, both the pulp and the seeds, which are believed to have vermicidal properties due to the content of compounds such as cucurbitacin and cucurbitin, which have been used for centuries in both human and animal care (Dotto and Chacha, 2020). According to Amin *et al*. (2019), Valdez-Arjona and Ramírez-Mella (2019), and Ait Nouisse *et al.* (2025), some bioactive compounds present in pumpkin seeds are promising therapeutic factors with antidiabetic, antioxidant, antitumor, and cytoprotective activities. Therefore, various pumpkin varieties are cultivated worldwide not only for the food industry, zootechnical uses, and decorative applications, but also for curative treatments. UV radiation has been considered for experimental studies in applied genetics for plant breeding, crossing irradiated and non-irradiated plants to obtain varieties with better properties. Other researchers (Kamal, 2023) have worked with hybrids derived from crosses between irradiated and non-irradiated summer squash plants, which have been shown to produce a greater number of fruits as well as larger and heavier fruits per plant. The mechanisms underlying these effects are attributed to chromosomal changes that can lead to positive effects for moderate or short irradiation times, depending on the plant species and irradiation conditions.

In our experiments, we looked for some bioeffects of UV-C on pumpkin seedlings grown from germinated seeds irradiated for up to several hours.

2. MATERIALS AND METHODS

2.1. Biological material

Healthy seeds were selected from a single pumpkin plant (*Cucurbita pepo* L. var. Butternut) to ensure a uniform genetic background; they were carefully cleaned and allowed to germinate on moistened porous paper in Petri dishes in the dark and at constant temperature (21.0 ± 0.5°C). Two sets of Petri dishes, each containing 20 seeds, arranged on moistened porous paper to constitute an irradiation sample, were exposed under identical conditions to UV-C radiation after germination.

2.2. UV-C exposure

To study the UV-C effects on seeds, we designed an experimental irradiation model. A Philips germicidal lamp with a total emission power of 50 W and 12 W in the UV-C range (254 nm) was used as an ultraviolet radiation source. Freshly germinated seeds in their Petri dishes were placed under the center of the lamp at a distance of 25 cm for 1-2-3 h, respectively.

Radiation doses were calculated using the Keitz formula (Sasges *et al*., 2012):

$$I = P\frac{2\alpha+\sin 2\alpha}{2\pi^2 DL}, \tag{1}$$

where the radiation power density or irradiance, I (W m^{-2}), resulted as 8.28 W m^{-2} at the distance D under the UV lamp center, and 2α is the angle at which the tube is seen from the center of the sample. For the seeds exposed in a Petri dish with 0.09 m diameter, we obtained incident UV-C energy doses ranging between approximately 0.19 J and 0.57 J for the irradiation time between 1 and 3 h. The results of applying the Keitz formula are considered reliable, with an accuracy of ±5% compared to radiometric measurements (Lawal *et al.,* 2008). This irradiance level is close to that reported by Barco *et al.* (2024), *i.e*., 8.18 W m^{-2}, in a cytogenetic study of UV irradiated onion as an alternative to animal models.

The control sample was not irradiated, but was stored and handled under the same conditions. When roots were about 1.5-2.0 mm long, tissue aliquots were collected for microscope studies. Seven-day-old seedlings were examined from a morphophysiological viewpoint and investigated with biochemical methods.

2.3. Cytogenetic investigation

After the irradiation time, all Petri dishes were maintained under the same conditions as before germination, *i.e.* in the dark and at a constant temperature (21.0 ± 0.5°C), for another 24 h. This is an absolutely necessary period to allow the cells to complete at least one more cell cycle, in which potential DNA repair processes can fix the damage, and the formation of micronuclei and chromosomal aberrations, as persistent damage, can occur (Barco *et al*., 2024). The next step was to fix the biological material in Carnoy's solution (absolute ethanol and absolute acetic acid in a 3:1 ratio) at room temperature up to 24 h. The role of this fixative is rapid penetration, preserving cellular structure, halting the cell cycle, and improving nuclear/chromatin staining. The samples were then placed in 70% ethyl alcohol and stored in a refrigerator (4±1°C) until proceeding for microscopy. For softening, the root tissue samples were placed in an HCl solution (absolute HCl and distilled water 1:1 ratio) for 8 minutes at room temperature, and then they were stained with modified carbol-fuchsin dye under cold conditions (4°C) for 24 h. For microscopic examination, the slides were prepared in a drop of 45% acetic acid using the squash technique (Singh, 2018). The acetic acid aids to macerate the tissue and disperse the cytoplasm, while the gentle pressure onto coverslip spreads the cells. Microscopic images were captured using a Nikon Eclipse 600 optical device and a Nikon Cool Pix 950 digital camera at a resolution of 1600×1200 dpi. Cytogenetic analysis aimed to identify cells with aberrant mitoses in the irradiated samples.

2.4. Biochemical assays

Biochemical analyses focused on two enzymes representative of antioxidant activity, superoxide dismutase (SOD), which has an important role in the defense against oxidative stress (Singh, 2018), belonging to metalloenzymes and catalyzing the conversion of superoxide radicals to hydrogen peroxide, and catalase (CAT), which catalyzes the dismutation of hydrogen peroxide into water and oxygen. SOD activity was assayed using the Winterbourn method (Stephenie *et al.,* 2020). The results were expressed as SOD activity units per gram of plant tissue. CAT activity was assayed according to Hadwan *et al.* (2024), and the results were expressed as units of CAT activity per gram of plant tissue. Protein content was assayed according to the Bradford method (Rekowsky *et al*., 2021), and the results were given in mg of protein per gram of green tissue. Malonylaldehyde (MDA) content, which is an indicator of lipid peroxidation, was assayed from the reaction with thiobarbituric acid (TBA), and the absorbance of the TBA-MDA complex was measured at 532 nm (Hodges *et al.,* 1999). Photosynthetic pigment content was calculated from the absorption spectra of ethanolic extracts from aliquots of green tissue according to Lichtenthaler and Buschmann (2001):

$$Chlorophyll\ A = V\,\frac{12.21E_{663} - 2.81E_{645}}{1000w}, \qquad (2)$$

$$Chlorophyll\ B = V\,\frac{20.13E_{645} - 5.03E_{663}}{1000w}, \qquad (3)$$

$$T.C. = V\,\frac{1000E_{470} - 3.27ChlA - 104ChlB}{1000w227}, \qquad (4)$$

where E_{663}, E_{645}, and E_{470} are the light absorbance values at certain wavelengths, V and w are the volume of the extraction solvent (ethanol) and the mass of the green tissue, *Chl A* and *Chl B* are the chlorophyll contents, and *T.C.* is the total carotene content. The morphophysiological study focused on the roots and stems of 7-day-old pumpkin seedlings, their lengths being measured with a millimeter ruler. The representation of the root and stem size histograms with the box-plot method was used for comparative discussions.

2.5. Statistics

A one-way ANOVA was conducted to assess the statistical significance between the control group and samples exposed to UV-C. Also, Tukey's Honest Significant Difference (HSD) post-hoc test was applied to explore pairwise differences among treatment groups. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Results of cytogenetic investigation

Some examples of abnormal mitotic cells are presented in Fig. 1. These images have highlighted the presence of chromosomal aberrations in root meristem mitotic cells for the irradiated samples; all expected modifications being found in ana-telophase and metaphase.

The illustration of normal stages of mitosis in pumpkin root cells is given for comparison, such as anaphase - with complete separation of sister chromatids and metaphase - with centrally aligned arrays of duplicated chromosomes ready for the formation of daughter cells (Fig. 1a, b, c), while the abnormal divisions are exemplified in Fig. 1d-l. Single and multiple bridges were identified in ana-telophase (Fig. 1d, e) and multipolar ana-telophase (Fig. 1f), which prevented correct separation and possibly led to diploid cells if repair mechanisms did not prevent this. Laggard and expelled chromosomes, representing parts of the genetic material that remained stuck in the cytoplasmic

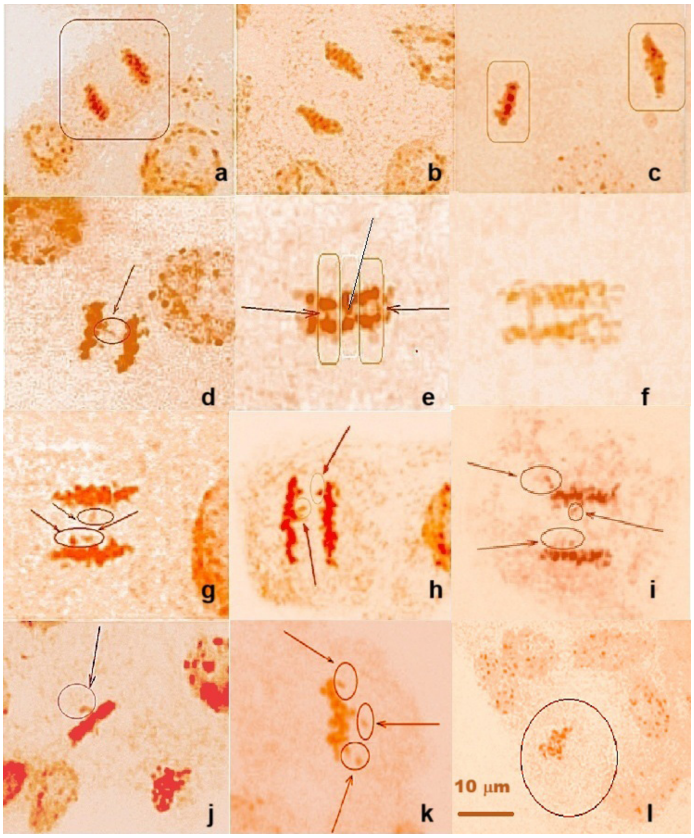

Fig. 1. Aspects of mitosis in pumpkin root meristems: a) normal ana-telophase; b) normal telophase; c) normal metaphase; d) ana-telophase with a chromosomal bridge; e) incipient ana-telophase with multiple bridges; f) multipolar ana-telophase with bridges; g) ana-telophase with three lagging chromosomes; h) ana-telophase with two lagging chromosomes; i) ana-telophase with lagging and expelled chromosomes; j) metaphase with one expelled chromosome; k) multiple expelled chromosomes in metaphase; l) C-metaphase.

space without migrating to the nuclei of the daughter cells (Fig. 1g, h, i), were found in abnormal ana-telophases and abnormal metaphases (Fig. 1j, k).

Figure 1 shows a C-metaphase, caused by disruption of the normal formation of the mitotic spindle such that the chromosomes are completely dispersed in the cytoplasm. Following the analysis of the microscope slides, no aberrations were observed in the control samples, nor was there any specific correlation between the type of chromosomal aberration and the exposure time in the irradiated samples. Other authors, such as Çavuşoğlu *et al.* (2022), who analyzed the effects of chromosomal changes induced by ultraviolet rays at the molecular level, found that chromosomal bridges and chromosomal breaks can form in UV-irradiated cells, following the dimerization of DNA pyrimidines, which blocks replication and correct transcription. These authors highlighted the presence of vagrant and sticky chromosomes, chromosomal bridges, chromosome fragments, and multipolar anaphases after irradiation of *Allium cepa* bulbs with UV-A and UV-C light up to 12 h.

Dunkern and Kaina (2022) studied the effects of UV-C radiation on *Allium cepa*, highlighting the presence of various chromosomal aberrations, including C-metaphase, chromosomal bridges, and delayed chromosomes, which denoted the genotoxicity of ultraviolet radiation for treatment times of tens of minutes. Also, Barco *et al.* (2024), who studied the exposure of onion to UV-C radiation with 0.818 mW cm^{-2} irradiance, reported the occurrence of chromosomal bridges, chromosome fragments, and lagging chromosomes.

3.2. Results of biochemical assays

Representative antioxidant enzymes, namely SOD and CAT, were tested in 7-day-old pumpkin seedlings grown from seeds exposed to UV-C along with seedlings grown from control seeds. SOD activity was generally found to be reduced up to 80% for a 3-h exposure time. An exception was highlighted for the sample exposed for 1 h, where an increase of approximately 20% was found (Fig. 2), which is consistent with the results reported by Kamel *et al.* (2022), who found an increase in antioxidant enzyme activity in spices for a relatively short time of UV exposure, followed by a decrease at longer exposures.

For all analyzed parameters, the ANOVA single-factor test revealed significant differences (three replicates for each biochemical assay were performed).

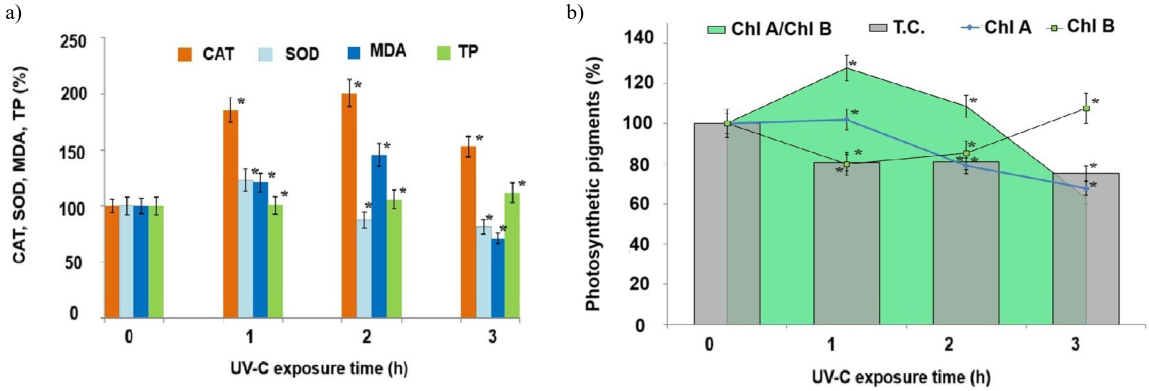

Fig. 2. Results of biochemical assays: a) catalase activity (CAT), superoxide dismutase activity (SOD), malondialdehyde content (MDA) and total protein content (TP) in seedlings grown from irradiated seeds; average values normalized to the control sample, n = 3; b) contents of photosynthetic pigments in the studied seedlings (Chl A– chlorophyll A content, Chl B – chlorophyll B content, T.C. – total carotene content, Chl A/Chl B – ratio of chlorophyll contents), n = 3; * marks statistical significance with p < 0.01 according to the ANOVA single factor test between groups compared to the control.

It can be assumed that the •OH radicals resulting from the photolysis of water on the surface of the germinated seeds penetrated the cells, triggering the generation of other toxic products, such as the superoxide radical and hydrogen peroxide molecules capable of promoting chromosomal modifications, some of which possibly affected the biosynthesis of enzymes. The reduced activity of SOD could be caused by the negative effect of UV-C on its biosynthesis in plant cells through certain genetic changes induced in the irradiated embryos. It is assumed that the reduced action of SOD on its substrate, the superoxide radical (O_2^-), leads to a change in the balance between free superoxide radicals and hydrogen peroxide molecules, with a higher amount of undecomposed O_2^- versus a lower production of hydrogen peroxide (Eq. (5)), having an indirect positive effect on the complex defense mechanisms against oxidative stress with a reaction rate of $2.4\times10^9\ M^{-1}\ s^{-1}$ (Scandalios, 1993):

$$O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2. \qquad (5)$$

Also, spontaneous dismutation of O_2^- occurs with a reaction rate of $\sim10^5\ M^{-1}\ s^{-1}$ (McCord, 1999). Since enzymatic activity is expressed relative to protein content, the total soluble protein content was presented. The results of the Bradford assay (Rekowsky *et al*., 2021) showed that, for all irradiated samples, there was an increase in protein content, with a constant progressive dependence on the duration of irradiation (Fig. 2a). This suggests a positive and sustained influence of UV-C radiation on the biosynthetic processes of total cellular proteins. In this context, however, catalase antioxidant activity was increased for all exposure times, showing that CAT biosynthesis was distinctly stimulated. The increase in catalase activity let us assume that hydrogen peroxide, the CAT substrate, was significantly decomposed in all irradiated samples, preventing its action of lipid peroxidation. Thus, this process allows seedlings grown from seeds exposed to UV-C to cope with the toxic product of oxidative stress, *i.e*., hydrogen peroxide (Eq. (6)) with a reaction rate $k=1.7\times10^7\ M^{-1}\ s^{-1}$ (Scandalios, 1993):

$$2H_2O_2 \rightarrow O_2 + 2H_2O. \qquad (6)$$

The sensitivity of plants to radiation is expected to depend on the species, as well as other factors such as the stage of embryo growth, irradiation time, *etc*. Thus, in other experiments, with onion bulbs, the researchers found that both SOD and CAT activity were enhanced following exposure to UV-C for 72 h, as well as MDA content (Çavuşoğlu *et al*., 2022).

Malondialdehyde (MDA) is known as a reactive molecule generated as a result of lipid peroxidation, which forms covalent adducts with proteins and DNA, leading to cellular damage. We observed an increasing trend of variation in MDA, so it seems that lipid peroxidation is remarkable even with increased CAT activity, which means that possibly undegraded superoxide radicals (due to reduced SOD activity, mainly for longer UV exposure times) could have a considerable contribution to lipid peroxidation in plant cells.

The Tukey HSD post hoc analysis ($\alpha = 0.05$) revealed distinct, parameter-dependent responses to UV-C irradiation, with antioxidant enzyme activities showing differential responses. Superoxide dismutase activity differed only in the 1-h samples compared to those at 2 and 3 h, with no other significant differences. Catalase activity did not differ significantly between 1 and 2 h of exposure, whereas all other comparisons revealed significant differences. MDA and total protein levels were statistically non-significantly changed after 1 h of UV-C exposure compared to the control, whereas longer exposures (2 and 3 h) differed significantly from both 1-h and control treatments.

Although CAT activity increased by approximately 80, 100, and 50% respectively after UV-C exposure for 1-2-3 h, SOD activity, after an increase of approximately 20% at 1 h, subsequently decreased by up to 20% at 3 h, which may indicate translational or genetic damage rather than adaptation to irradiation.

Since biochemical analyses describe the situation of seedlings grown from irradiated embryos, the most credible source of the observed changes should be the genetic changes exhibited by irradiated embryonic cells and expressed in the biochemical changes in seedling tissues. However, the repair mechanisms with which cells are equipped could remedy some of the genetic abnormalities. The bioeffect of seed exposure to UV-C was also observed at the biochemical level in photosynthetic pigments (Fig. 2b). Chlorophyll A biosynthesis appeared to be reduced by 25-30% for 2- and 3-h irradiation times, and a reduction in chlorophyll B biosynthesis was present for 1- and 2-h UV-C exposure - by 20 and 15%, respectively. According to the Tukey HSD post hoc analysis, the photosynthetic pigment parameters exhibited significant differences among all irradiation times, revealing distinct temporal response patterns for chlorophyll A, chlorophyll B, total chlorophyll content, and chlorophyll ratio.

Also, Kumar and Pandey (2017) evidenced a reduction of photosynthetic pigment contents in seedlings grown from UV irradiated seeds of *Choriandrum*, which also showed numerous chromosomal aberrations. The results are consistent with those reported by Sebastian *et al*. (2018), who revealed low chlorophyll content in seven-day-old plants grown from UV-irradiated fenugreek seeds, a plant species used for both food and medicinal purposes. We also found a trend of decreasing the concentration of carotenoid pigments (Fig. 2b) and, as a result of the decrease in their biosynthesis in plant cells, chlorophylls might have been less protected against photo-oxidative damage (Kumar and Pandey, 2017).

However, the most important result seems to be the increase in apparent photosynthesis efficiency by almost 30% for the shortest exposure time of 1 h, as evidenced by the ratio between chlorophyll A and chlorophyll B.

3.3. Results of morphophysiological study

Some representative images of the analyzed seedlings are given in Fig. 3. Statistical representation with the box-plot method was applied for root (Fig. 4a) and stem lengths (Fig. 4b) of the 7-day-old pumpkin plantlets.

The compact clustering of the data was highlighted in the 25-75% range, according to the statistical box-plot representations. The root lengths appeared less different for the irradiated samples compared to the control sample, the

a) b)

Fig. 3. Pumpkin seedlings: a) plantlets grown for seven days in Petri dishes, b) stems and roots.

a) b)

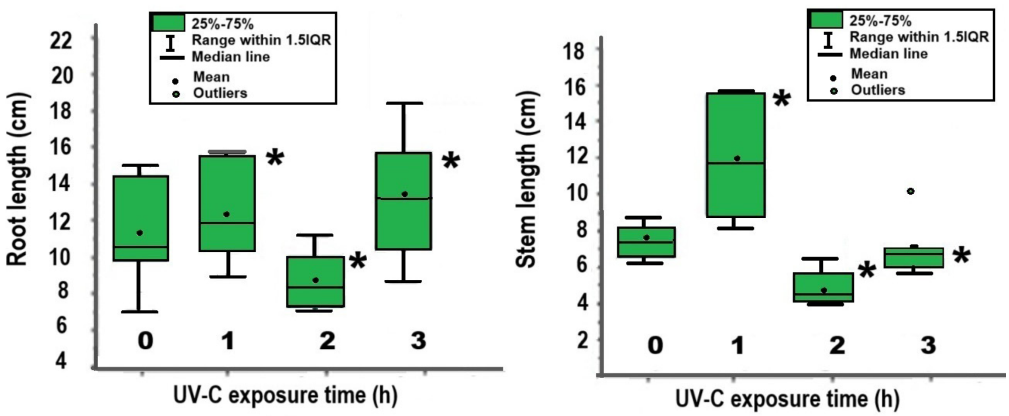

Fig. 4. Box-plot comparative representation of root and stem lengths (n = 20): a) root length statistics in the pumpkin seedlings, b) stem length statistics in the pumpkin seedlings, *statistical significance with $p < 0.01$ according to the ANOVA single factor test between groups compared to the control.

box-plot showing only slightly higher values for a UV-C exposure time of 1 h (10% for the median value), although they had significantly lower values for 2 h of irradiation – by about 30% (Fig. 4a). For the seedling stem length, the centered positions of the median values suggested symmetrical histograms.

An almost two-fold increase compared to the control sample for the seedling stem length was obtained for an exposure time of 1 h (in concordance with the results for corn seedlings reported by Garbeles *et al.* (2024)), while the values shifted towards shorter length values for irradiation times of 2 and 3 h (Fig. 4b).

The stimulatory effect of radiation for a short exposure of 1 h seems to reflect fairly correlated results between photosynthesis efficiency and seedling stem length.

The post hoc Tukey HSD statistical analysis for samples of 20 seedlings showed that stem length was mainly affected after 1 h of exposure, this sample being significantly different from the control and the 2- and 3-h irradiation groups. Root length showed a maximum response at 2 h of irradiation, differing significantly from both 1-h and 3-h exposure.

It could be said that the decrease in total carotene content reduces antioxidant protection on chlorophylls, which thus also decrease, especially chlorophyll A. The increase in photosynthesis efficiency at 1 h of exposure, when both SOD and CAT have values above those of the control, is reflected in the remarkable increase in stem length in seedlings.The complexity of the response of embryonic cells to UV-C radiation seems to be only partially resolved by basic cell biology tools, mainly because DNA damage could be partially erased by enzymatic repair mechanisms.

4. CONCLUSIONS

Preliminary observations of the response of pumpkin seeds to UV-C light under controlled laboratory conditions revealed some notable changes for irradiation times of 1-2-3 h. Chromosomal changes were identified in ana-telophase and metaphase, catalase activity was increased by up to 100%, but SOD activity was generally low. Additionally, the low carotene content observed was linked to a decrease in chlorophyll A levels, but with an increased chlorophyll A/B ratio, while root and stem length showed a statistically greater increase for the 1-h irradiation time. It is possible that some chromosomal aberrations can propagate to cultivated plants, which could form the basis of biotechnological benchmarks for the reproduction of mutations obtained with appropriately adjusted artificial sources of ultraviolet radiation. Increasing the biosynthesis of some antioxidant enzymes and the efficiency of photosynthesis, for relatively short-term irradiation of one hour, could promote seedling growth. Overall, the results indicate that UV-C irradiation induces oxidative and genotoxic stress responses in *Cucurbita pepo* during early developmental stages, with certain physiological responses being partially mitigated at short exposure times. These findings should be regarded as preliminary observations obtained under controlled laboratory conditions, and any extrapolation to long-term plant performance or agronomic applications requires further research.

Conflict of interest. The authors declare that there is no conflict of interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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