ORIGINAL PAPER

SPECTRAL AND MICROSCOPY STUDY ON UV-C RADIATION BIOEFFECTS IN SOME VEGETAL ORGANISMS

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Abstract. *The experimental study was focused on the effects of ultraviolet radiation in maize (Zea mays) at both cytogenetic and metabolic levels. Caryopses with uniform genophond were let to germinate in INCUCELL room in controlled environmental conditions before exposure to UV-C radiation for two different time durations. Biochemical investigation carried out by spectrophotometric method showed that photosynthesis pigments in seedling green tissue was lowered with 30%-40% denoting cell metabolism perturbation because of UV exposure during plant early ontogenetic stages. Several main types of chromosomal changes were highlighted in root meristem tissue - namely micronuclei and chromosomal aberrations: expelled and lagging chromosomes, inter-chromatin bridges – totalizing 4 to 4.5 % of total analyzed cells which suggested DNA injuries in developing plantlets.*

Keywords: *UV-C exposure, Zea mays, chlorophyll ratio, chromosomal aberrations*

1. INTRODUCTION

Plant responses to the impact of UV radiation may include changes in physiological, morphological and genetic features depending on the plant species and age and also on the UV radiation intensity and wavelength range.

UV radiation spectrum is known as formed of three parts or subdomains. UV-A domain ranges within 320-400 nm and represents the dominant part of all UV rays incoming from solar emission therefore being responsible for largest number of bioeffects observed in the biosphere components. UV-B domain ranging within 280-320 nm has three times lower weight than UV-A in the solar spectrum but it has more diversified bioeffects array in plants according to Hollosy, 2002 [1].

The UV-C domain (200-280 nm) is extremely harmful to living organisms; although even at present time under natural conditions of solar irradiation the UV-C seems not to be an actual threatening however the balance could change from atmospheric causes as those related to ozone issues or from cosmic outcomes. Since vegetation is recognized as a reliable screen protecting human population against many harmful physical and chemical factors the study of UV radiation influence on plants has involved multidisciplinary approach with increased interest for experimental laboratory study.

In pepper seedlings (*Capsicum longum* A.DC.) grown in controlled laboratory room (Hosseini *et al*., 2011, [2]) shoot growth decreased significantly in UV-C-exposed plants as well as their leaf area. As plant growth is based on photosynthesis *and specific assimilatory* pigments, the UV-C absorption in chlorophylls and carotenes is also of particular interest.

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It seems that UV light can be captured partially in the chlorophylls molecules as shown by their absorption spectra but it is important also the radiation energetic impact in the amino acids (absorbing only in UV range), and thus in the proteins that form complexes with chlorophylls or have enzyme role in chlorophyll biosynthesis.

UV-C treated plants of savory (*Satureja hortensis L.*) grown and irradiated in laboratory showed a significant decrease in chlorophyll A, B and total and carotenoid contents as reported in [3]. Also chlorophyll degradation was reported in Chinese kale (*Brassica*) following UV-C treatment [4]; in [5] it was found that UV radiation caused a reduction in plant growth and photosynthetic capacity while the effects in assimilatory pigment levels were reported also in [6]. It is accepted that complex interaction occurs between the UV light absorbing molecules resulting in their informational exchange that may lead eventually to signals interfering with cell physiological functions [7]. However, the mechanisms by which these photoreceptor molecular complexes generate many diverse physiological responses still represent a challenge for the scientific research [8].

Effects of ultraviolet radiation on plants vary both with species (the woody species are more resistant to radiation than herbaceous ones) and varieties of the same species. It was evidenced that in the irradiated bean plantlets (*Phaseolus vulgaris* L.), the frequency of chromosomal aberrations increased with the decrease of radiation wavelength (from UV-A, to UV-B and to UV-C) but differs from one cultivar to another [9]. Also in barley the UV-C induced different cytogenetic changes were found to depend on the cultivar [10].

DNA seems to represent a major target of UV-B and UV-C rays since nucleic acid biomolecules absorb preferentially in this spectrum range so that, when repairing enzymes cannot compensate the induced molecular damages [11] various DNA photoproducts can result that may cause mutations during replication [12].

The results reported in [13] showed that three days old corn seedlings were exposed to 6.2–9 kJ/m^2 UV-C radiation (the dose rate was 6.2 W/m^2) which resulted in mitosis rate perturbation and increased frequency of chromosomal aberrations. Meristematic root cells of *Crepis capillaris* were found also very sensitive to UV-C exposure as resulted from chromosomal aberration screening and counting according to [14].

In the present work the cytogenetic analysis and assimilatory pigment assays were carried out to evidence maize seedling responses to UV-C exposure. Also cytogenetic investigation was accomplished since the stability of cell division and genetic structures are important for plants development.

2. MATERIALS AND METHODS

2.1. Biological Material

The study was developed on seeds and seedlings of maize (*Zea mays* conv. *dentiformis* Korn., early hybrid *Suceava 108* [15]. The source was the Agricultural Research and Development Station in Suceava, Romania).

Three series of maize caryopses samples, each consisting in 50 caryopses of *Zea mays*, harvested from a single genitor plant (in order to ensure uniform genophond) were arranged for germination in Petri dishes on moistened filter paper in an INCUCELL room for 2 days (in darkness and constant temperature of 21.0 ± 0.1°C); pure distilled water was supplied to the young plants during the whole experiment.

2.2. Exposure to UV-radiation

Following germination, the plantlets were exposed to UV-C light using as radiation source a UV-C lamp (254 nm, 30 W) at 50 cm high above the caryopses plane (i.e. the bottom of the Petri dishes).

2.3. Microscopy Investigation on UV-C Effects

The effect of UV-C radiation on meristematic root cells of maize caryopses was investigated following exposure to UV-C that begun every day at 8:00 a.m. and lasted for three consecutive days. Two sets of samples were prepared: 2 hours daily irradiation and 4 hours daily irradiation starting from the first day of germination.

Control samples were prepared in the same way being kept in the INCUCELL room, in darkness at constant temperature 21.0±0.1 °C, in order to favor germination - except UV-C exposure wasn't applied after that.

Microscopy investigations on the root meristematic cells were carried out when first root tips reached the length of maximum 10 mm; the tissue aliquots were prepared for chromosome visualization by applying Feulgen rapid staining method [16]. Counting of normal and aberrant dividing cells in interphase as well as the cells frozen in various stages of mitotic division (prophase, metaphase, anaphase and telophase) was carried out on 10 microscope fields for each microscope slide of controls and UV-C exposed samples. Optical microscope NIKON Y-FL eclipse e600 assisted by photo camera NIKON e950 were used for slide screening. The genotoxic effects of UV-C irradiation were quantitatively estimated by calculating the mitotic index (M.I.) and the percentage of chromosomal aberrations (aberration index, A.I.).

2.4. Biochemical Assay of Assimilatory Pigments

To investigate metabolic changes in green tissue further irradiation was done for five more consecutive days by applying the same experimental protocol.

The contents of chlorophyll A (chll A), chlorophyll B (chll B) and total carotene pigments (t.c.) in the green tissues of maize seedlings were assayed spectrophotometrically based on 85% acetone extracts. From each sample, about 0.2 g fresh green tissue was crushed in a mortar with acetone solution. Photosynthetic pigment extracts were investigated based on light extinction recorded using the Shimadzu UV-1700 spectrophotometer in the visible range, in 1 cm quartz cells, at the wavelengths of 663 nm, 645 nm and 470 nm, accordingly to calculation method of Lichtenhaler and Welburn [17].

2.5. Statistical analyses

Statistical analysis was accomplished by carrying out five repetitions of the experimental measurements and calculating average values and standard deviations while Student *t*-test was applied for the statistical interpretation of the results; the differences between the UV-C irradiated samples and control ones were evaluated relatively to the significance threshold *p* of 0.05.

3. RESULTS AND DISCUSSION

The graphs (Figs. 1-4) for assimilatory pigment levels allowed analyzing the influence of UV-C exposure upon young seedlings. In Fig. 1 an obvious decreasing (with 31%, $p<0.05$) of chlorophyll A was shown for the seedlings corresponding to 2 hours daily irradiation compared to the control samples, while for the seedlings corresponding to 4 hours daily irradiation, still more evident inhibitory influence can be seen (chlorophyll A level decreased with 48%) comparatively to the control; standard deviation was of about 9% ($p<0.05$).

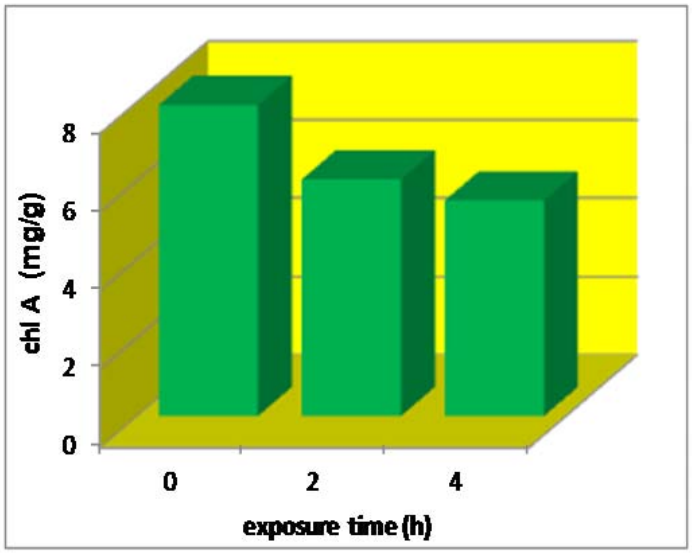

Figure 1. Chlorophyll A level (Chl A in mg pigment per gram of fresh tissue) in seedlings grown from embryos exposed to UV-C.

Similarly changes have been observed in *Phaseolus vulgaris L*. by the Y. Kara from Turkey [18]. The exposure to UV-C rays adversely affected the growth and photosynthesis in the bean plant. The plants were raised in an automated green house with the ecological conditions. He observed that seedling height was reduced by the exposure of UV-C. Total chlorophyll content significantly decreased in the UV-C exposed plants. Similarly, chlorophyll A and B were also significantly decreased in the leaves of plants exposed to UV-C in comparison with the control plants.

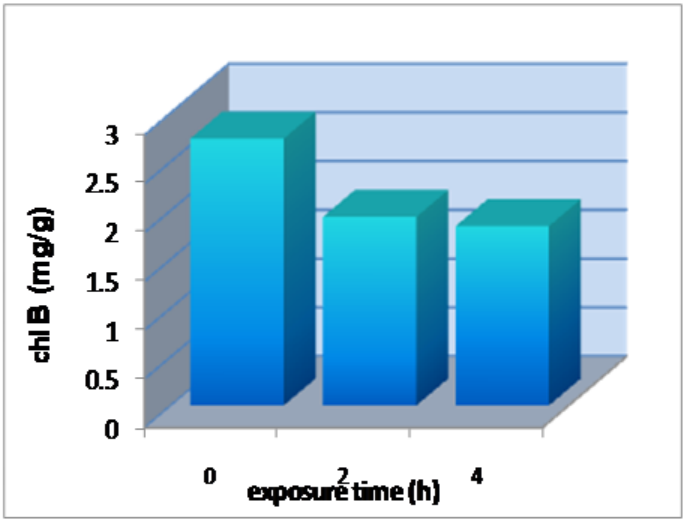

Figure 2. Chlorophyll B level (Chl B in mg pigment per gram of fresh tissue) in seedlings grown from embryos exposed to UV-C.

The changes in the chlorophyll B - Fig. 2 and total carotene pigments - Fig. 3 have revealed similar variations. Chlorophyll B was diminished with about 30% and respectively 36% in the two types of exposed samples ($p<0.05$, Fig. 2). Carotene pigments level was diminished with about 30% and respectively 37% for 2 hours and respectively 4 hours of UV-C irradiation ($p<0.05$, Fig. 3). It can be assumed that the effect of exposure to UV-C has led to significant decrease in chlorophyll B and carotene pigments although slightly different compared with chlorophyll A decrease. Thus one can say that metabolic changes in the investigated seedlings occurred as consequence of UV-C exposure of maize caryopses even if the biochemical assays carried out could not suggest the underlying metabolic pathways.

The observed reduction in leaf length is a common response to UV-A and UV-C and this has been demonstrated in a variety of species including *Satureja hortensis L.* UV-C-treated plants showed a significant decrease in shoot growth, leaf number and shoot fresh and dry weights as well as leaf protein, leaf carbohydrate, chlorophyll A, B and total and carotenoid contents [3]. The content of chlorophyll A, chlorophyll B and chlorophyll sum decreased in UV-C exposed samples. Only the reduction of chlorophyll B in UV-A treated plants was not significant. Carotenoid concentration was also reduced in UV-C exposed plants and this reduction was significant.

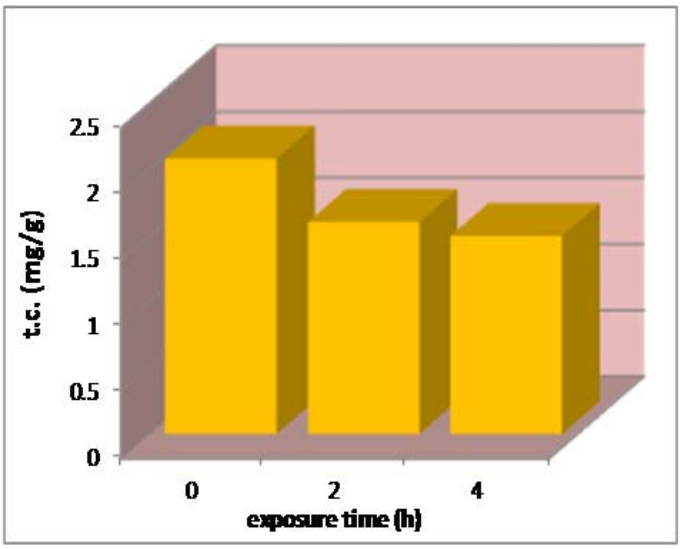

Figure 3. Total carotene (t.c.) pigments (in mg pigment per gram of fresh tissue) in seedlings grown with exposure to UV-C.

As presented in Fig. 4 the ratio of the two chlorophylls concentrations exhibited no significant variations ($p>0.05$) among control and irradiated samples. It is known that chll A/chll B ratio could be taken as indirect indicator of the efficiency of energetic processes from photosynthesis system PSII located in chloroplast membranes (the apparent efficiency of photosynthesis), which therefore determines the plantlet development and growth. Several experiments reported in literature showed negative biological effects of UV-C radiation on plants; for example Hosseini and coworkers [2] conducted a study to evaluate the effects induced by ultraviolet radiation on photosynthesis pigments in pepper plants (*Capsicum longum L.*) grown in greenhouses. It was found, that in the exposed samples, both chlorophyll A and chlorophyll B contents have slightly decreased, although the reduction was not significant while total chlorophyll content was diminished significantly in the plants exposed to UV -C radiation.

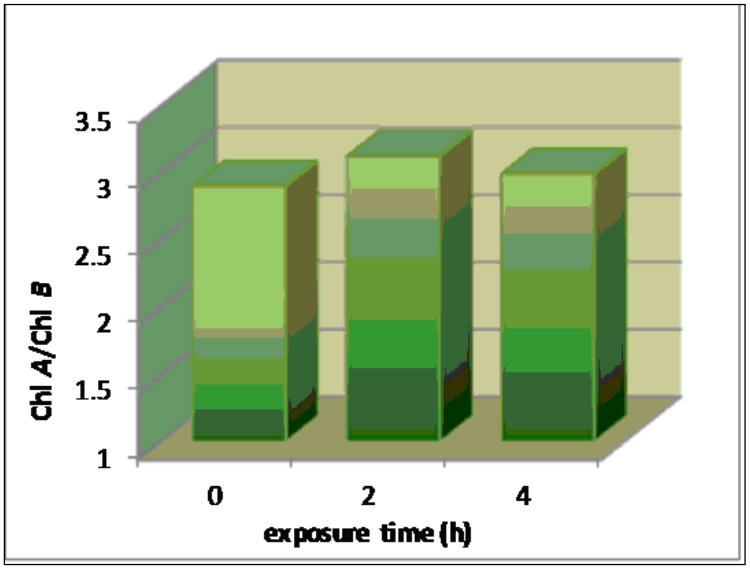

Figure 4. The ratio of chlorophyll A and chlorophyll B content (Chl A/Chl B) for the three types of samples.

Other report [4] mentioned that chlorophylls were found diminished in *Brassica* mature individuals exposed to UV-C radiation. Previous study [19] has found that UV-C treatments delayed yellowing, chlorophyll A and B degradation in broccoli florets. Broccoli heads were irradiated with different doses of UV-C light (4, 7, 10 or 14 kJm^{-2}) and then stored at 20 °C for 5 days to accelerate senescence. It was noted that although chlorophyll degradation was highly delayed after the treatment at 14 kJm^{-2}, no difference in the hue angle value was found.

Further qualitative and quantitative investigation of cytogenetic changes induced by UV-C irradiation in the present study was based on the analysis of microscopy raw data.

Qualitative investigation shown that the main types of chromosomal aberrations induced in meristem cells were: lagging chromosomes, expelled chromosomes, simple or multiple inter-chromatidian bridges and multipolar ana-telophase; in some cases the chromosomal aberrations were observed in association of two or three within the same root meristem cell. Also micronuclei were observed in some abnormal dividing cells. In Fig. 5 exemplification of normal and aberrant mitosis phases is presented for anaphase and telophase.

In other study, for example, Tiunaitiene *et al*. [14], have analyzed the influence of different physiologically temperatures on UV-C induced chromosomal damage and photoreactivation in *Crepis* root cells. The first abberations apeeared in the third hour after UV irradiation, most celles with chromatid-type aberrations (2.4%) being found in the fourth hour after UV exposure.

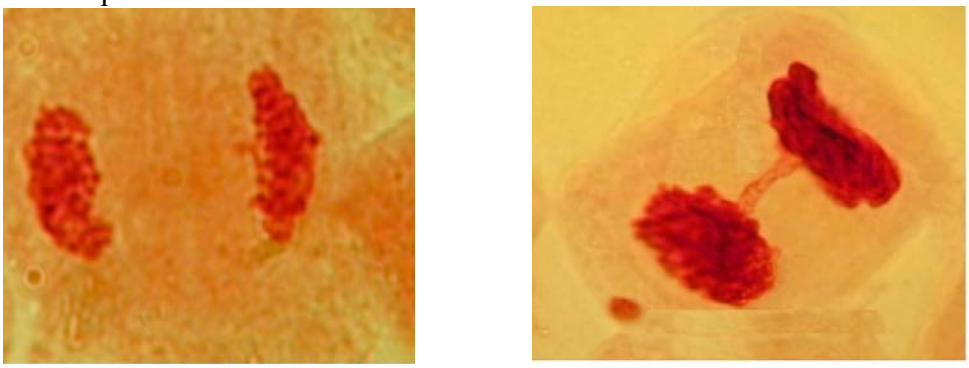

Figure 5. a) Normal mitotic division; b) inter-chromatidian bridge and micronucleus observed during cell mitosis.

Quantitative data processing provided the mitotic index expressing the percentage of dividing cells from total screened cells, and also the chromosomal aberration index representing the percentage of aberrant cell divisions related to the total dividing cells.

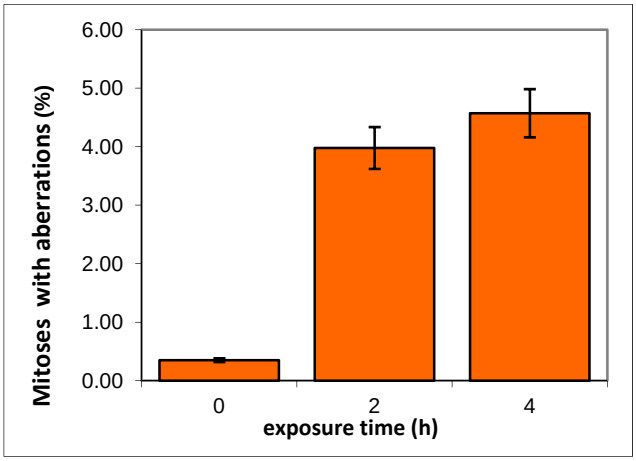

Figure 6. Frequency of cells with chromosome aberrations observed during cell division.

In Fig. 6, the graph of aberrant cell mitoses was plotted; remarkable difference between irradiated and control samples can be seen. The percentage of aberrant dividing cells i.e. cells presenting chromosome changes during mitosis was about ten times higher for two hours exposed samples than for the control ones (standard deviation was of about 9%); still higher percentage of chromosomal aberrations in four hours exposed embryos was estimated: 11 times higher than in controls (p<0.00001). This is undoubtedly the cytogenetic proof of remarkable UV-C genotoxic effect. In case of control sample still exists a small number of aberrations that could be induced by uncontrollable environment factors gradients like weak magnetic field variations or transient fluctuations of ionizing radiation background. Another report, that of Sokolova *et al*. [20] showed that the different exposure modes to acute UV-C and chronic gamma-irradiations had an influence on satellite DNA methylation pattern of corn seedlings inducing an increase of chromosome aberration yields. In this report UV-C exposure of seedlings from preliminary un-irradiated seeds with adaptive dose leads to increasing chromosome aberration yield whereas exposure of seedlings from preliminary irradiated seeds induced the hormetic effect. The mitotic index was relatively constant in control sample and UV-C exposed ones (Table 1) with no statistically significant differences (p>0.5), which let us conclude that UV-C has not influenced the rate of cell division meaning that neither detectable mitosis delay nor its stimulation occurred.

Table 1. The mitotic index in control and irradiated samples

Variant	Total analyzed cells	Total cells in interphase		Total cells in division	
	No.	No.	%	No.	%
Control	3147	2863	90.08	284	9.02
UV - C 2 h	3912	3560	91.00	352	9.00
UV - C 4 h	3851	3501	90.91	350	9.09

4. CONCLUSIONS

The assay of assimilatory pigments levels carried out in this work as well as the counting of chromosomal aberrations in root meristems allowed to formulate conclusions such as:

a. the biosynthesis of assimilatory pigments was affected as resulted from 20 to 40% diminution of chlorophyll and carotene pigment levels;
b. the ratio of chlorophyll A and chlorophyll B contents remained almost constant suggesting remarkable stability photosynthesis efficiency ratio against UV-C radiation.
c. the percentage of cells showing various chromosome aberrations following UV-C exposure was 10 to 11 times higher than in the control sample (unexposed) as resulted from aberration index values.

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