Romanian Reports in Physics **XX**, XYZ (2018)

# ON SOME GENOTOXIC EFFECTS OF UV-C RADIATION IN ROOT MERISTEMES IN *CUCURBITA PEPO* L.

A. VERDES-TEODOR[1], G. VOCHITA[2], D. CREANGA[1]

[1]University Alexandru Ioan Cuza, Physics Faculty, Biophysics&Medical Physics, Iasi, Romania, 11, Blvd. Carol I, e-mail: mdor@uaic.ro
[2]Institute of Biological Research, Iasi, 11, Blvd. Carol I, e-mail: gabrielacapraru@yahoo.com

*Received*

*Abstract*. The influence of high energy ultraviolet rays on radicular meristeme tissues of pumpkin plantlets in early ontogenetic stages was studied in the frame of laboratory experimental arrangement. Cytogenetic analysis was accomplished on adequately prepared single layer cells, colored with fuchsine reagent for chromosome selective visualization. Microscope screening on thousands of cells allowed the evidencing of changes induced by ultraviolet radiation at the levels of mitosis main phase that were comparatively analyzed for two exposure times to UV-C germicidal lamp, revealing dose-response behavior. The percentage of dividing cells as well as the percentage of abnormal divisions were counted and discussed based on tables and graphs generated with average values and standard deviation. Cytotoxicity of ultraviolet ray exposure was emphasized from both those cytogenetic parameters, raising the concern regarding biosphere adaptation to environmental occasional high gradients of radiation background.

Key words: UV-C radiation, pumpkin, mitosis, chromosomal aberration.

## 1. INTRODUCTION

Vegetation is naturally exposed to ultraviolet rays since those are a ubiquitous component of sun light, representing about 7-9 % of total radiation that reaches the biosphere, with higher impact on cellular structures than any other component of optical electromagnetic spectrum. As the atmospheric ozone layer seems to be no more a safe screen for plant cultures against UV constraints (because of ozone layer deterioration following anthropologic activities and cosmic events) [1], random UV impact on vegetation needs to be studied from various viewpoints [2-4], the putative perturbations on plant genome representing an obvious priority for monitoring biosphere sustainability.

Although not dominant in the sun radiation spectrum, UV-C rays, are released also from more and more numerous artificial sources like germicidal lamps, devices for microbiological improving of drinking water [5-6] and others, and became of interest once their energy was found to be preferentially absorbed by DNA molecules of surrounding organisms.

Since several decades new scientific preoccupation was related to UV-C bioeffects, for example with focus on the chromosomal aberrations induced in some common vegetable species like barley [7], or on the inhibition of anthocyanin production in sorghum internodes [8].

According to several studies [9-10] plantlets response to UV-C differs from that to the UV-B radiation at the level of tissues biochemistry and photosynthesys; the mentioned authors found opposite effects on carotenes levels, recorded for UV-C versus UV-B exposure. In the same time, sun light provides pathways to balance mutagenesis (basically, the lesions induced in the cellular DNA) and also cell death triggered by high energy UV rays, for example through the excision of damaged nucleotides and DNA repair by photolyase [11], i.e. photoreactivation, that enables the prevention of propagation to next generation of some genotoxic effects [12]; but this is valuable only when UV flux is not overwhelming living organism adaptation capacity. Recently UV-C exposure risk in humans was studied with focus on carcinogenesis [13]. Not only direct action of UV-C on DNA molecules was emphasized but also it was suggested that UV-C could cause as well indirect DNA damage because of generated ROS (reactive oxygen species) [14]. However pyridoxamine administration after UV-C irradiation was found effective in cancellation of UV-C-induced apoptosis by suppressing the ROS formation in human keratinocytes [15].

Plant sensitivity to UV exposure depends on the species (for instance woody species being more resistant than grassy ones), on the varieties belonging to the same species, and on the UV photons energy, as reported in [16], relatively to the chromosomal aberrations found in bean meristemes. In [17] the authors have also discussed different cytogenetic changes in different barley species. We analyzed the response of less studied agroindustrial plant, the pumpkin, following controlled UV exposure in laboratory experiment.

## 2. MATERIALS AND METHODS

### 2.1. BIOLOGICAL MATERIAL

Seeds of pumpkin (*Cucurbita pepo* L.), selected from single plant (to ensure uniform genophond of studied samples) were let to germinate in darkness and constant temperature (20.0±0.5 °C) in INCUCELL room, in 9.5 cm diameter Petri dishes on wet paper support.

### 2.2. UV-EXPOSURE

Immediately after germination, when up to 1.5 cm roots developed, the Petri dishes were let uncovered under the UV-C flow delivered by germicide source (50

W power, λ<280 nm) placed above at 50 cm height. Two exposure time durations were chosen, one hour and two hours respectively, for the two sample arrays (three Petri dishes each). Control sample array was kept in the same environmental conditions (room temperature of about 22 °C) except they were not irradiated.

### 2.3. CYTOGENETIC ANALYSIS

Aliquots from radicular meristemes were prevailed and immersed in adequate volumes of glacial acetic acid:ethanol (1:3 v/v) for 24 hours, being after that stored in 70% ethanol. To prepare microscope slides, the vegetal tissue aliquots were hydrolyzed (10 minutes in 50% HCl) to destroy cellulosic cellular walls; then nuclear material of cells was stained (Fuelgen method, with modified carbol fuchsin [18] by immersion in that reagent for 24 hours and storage at +4 °C; finally the radicular meristeme samples, characterized by selectivelly colored chromosomes, were withdrawn from fucsine reagent and squashed in 45% acetic acid [19] as single layers on glass supports (microscope slides). NIKON Y-FL eclipse e 600 light microscope was used to explore such prepared samples, by screening ten microscopical fields on each slide.

Quantitative cytogenetic parameters were estimated:

$$MI = \frac{Total.number.of.dividing.cells}{Total.number.of.counted.cells} \quad (1)$$

$$AI = \frac{Total.number.of.abnormal.mitosis.cells}{Total.number.of.dividing.cells} \quad (2)$$

Statistics. Statistical analysis was consistent with data processing from screening five slides for each experimental variant, and counting cells from ten microscope fields of each slide. Calculation of average values and standard deviations was accomplished for graphical plots of the results; student t-test was used to search for the statistical significance of the differences between the UV-C exposed samples and control ones (relatively to the significance threshold $p$ of 0.05).

## 3. RESULTS AND DISCUSSION

By surveying microscope slides, according to Fig. 1, the dividing cells were identified in the main mitosis phases (prophase, metaphase, anaphase and telophase) as well as in mitotic rest (interphase) (Fig. 1 a). In prophase (Fig. 1 b) the condensation of the two chromosome arrays occurs while the nucleus membrane disintegrates; in metaphase (Fig. 1 c) the chromosomes are concentrated in the central area of the mother cell; in anaphase (Fig. 1 d, e) the chromosomes

migrate toward the two poles of dividing cell and finally, in the telophase (Fig. 1 f), they form the two new nuclei of the future daughter cells.

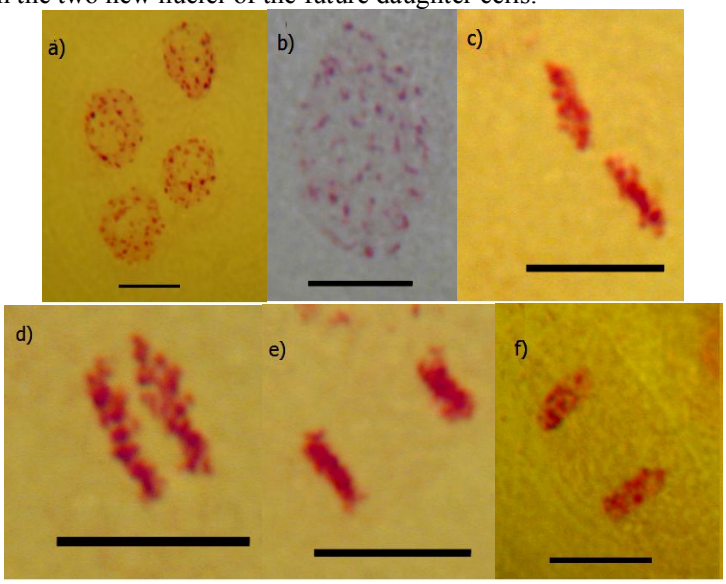


Fig. 1 – The normal stages in radicular meristem of *Curcubita pepo* L.: (a) – interphase; (b) – prophase; (c) – metaphase (two cells); (d) – incipient anaphase; (e) – ana-telophase; (f) – telophase. Bar = 10 µm.

Total numbers of cells found in different mitosis phases and interphase in Table 1 are given.

*Table 1*

Results of counting mitosis cells in the meristeme samples

| UV exposure time (h) | Total analyzed cells | Total interphase cells | Total mitosis cells |
|---|---|---|---|
| 0 | 5664 | 4908 | 756 |
| 1 | 6235 | 5440 | 795 |
| 2 | 5370 | 4719 | 651 |
| Total prophase cells | Total metaphase cells | Total anaphase cells | Total telophase cells |
| 523 | 108 | 63 | 62 |
| 506 | 133 | 81 | 75 |
| 406 | 93 | 79 | 73 |

In Fig. 2, the changes in the frequency of cells in the main mitosis phases are presented for UV exposed samples and control ones; standard deviation was of 8.5%.

One can see that in UV irradiated germinated seeds the percentage of cells in prophase is evidently reduced from about 9.2% in control samples to 8.1 % for one hour exposure and to about 7.6 % for two exposure hours ($p<0.05$), indicating the inhibition of cellular synthesis processes necessary to the triggering of next mitosis phase; the metaphase cells appears increased for one exposure hour while the anaphase and telophase cells seem to be slightly more numerous for two hours exposure to UV radiation.

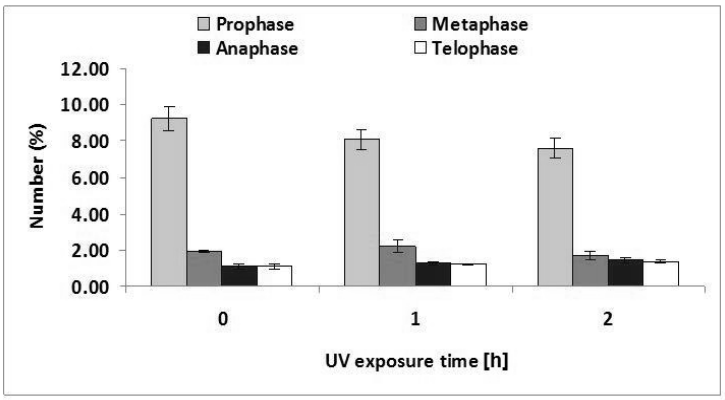


Fig. 2 – UV-C radiation influence on the mitosis phases.

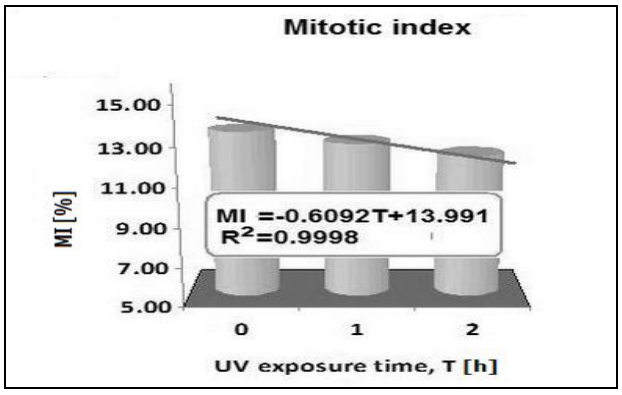


Fig. 3 – UV radiation effect on cell division capacity; MI-mitotic index.

The accumulation of cells in anaphase suggests an impairment of chromosome migration toward the cell poles while the increase of telophase cell

number could be associated with the delay or blockage of daughter cells separation. In Fig. 3 the mitotic index (MI) is represented by the summation of all cells in mitosis comparatively with the total number of counted cells in the studied samples (standard deviation of 7%). MI represents one of the most reliable parameter of cellular survival especially under the pressure of cytotoxic constraints given by environmental gradients of radiation, temperature, chemicals etc.

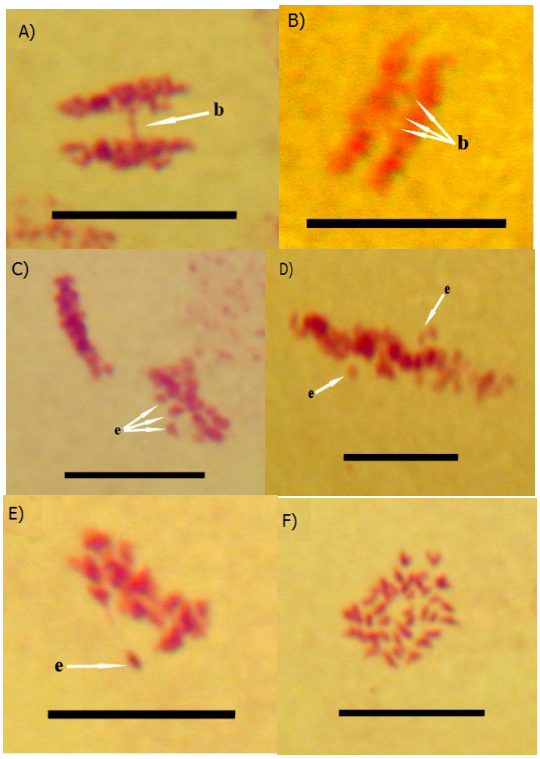


Fig. 4 – Abnormal dividing cells in meristemes of *Curcubita pepo* L. presenting the main types of chromosomal aberrations: A) ana-telophase with single interchromatidian bridge; B) ana-telophase with three bridges; C) metaphase with three expelled chromosome; D) metaphase with two expelled chromosomes; E) metaphase with single expelled chromosome; F) C-metaphase. Bar = 10 μm.

Linear fitting of the mitosis data (correlation coefficient of 0.999) provided the progressive decrease of dividing cell percentage with 0.6% per irradiation hour ($p<0.05$), directly proportional to the exposure time to UV rays, i.e. a dose-response behavior it was suggested. It means that for the analyzed experimental data, mitoclastic effect [19] of UV-C rays was emphasized, the sensitivity of mitosis to UV irradiation being describable through the regression line slope. The rate of mitotic index variation could be taken as genotoxicity impact indicator. We

mention that no aberrant divisions were found in the non-irradiated samples even this is not compulsory, considering putative uncontrolled environmental physical or chemical gradients in some cases.

Further we focused on abnormal divisions, with various chromosomal aberrations. Interchromatidian bridges, indicators of clastogenic action [20] of the genotoxic factor, in this case UV radiation, imped normal separation of the two daughter nuclei so that single descendant cell results with double chromosome array (a diploid cell, practically viable but bearing some changed, usually amplified phenotypic features). Lagged chromosomes remain along the divion axis and consequently one of the two daughter cells are going to miss some genetic information beared by retard chromosomes; expelled chromosomes represent also missing genetic information except they are spread laterally relatively to the division axis; C-metaphases are characterized by broken genetic material like in the case of colchicine reagent used for other types of experimental cytological studies. Lagged and expelled chromosomes as well as C-metaphases are known as indicators of aneugenic action [20] of genotoxic agent, meaning changes in the chromosome number in the daughter cells. Following peer analysis of recorded microscope images we have identified cells with one type of chromosomal aberration such as: Fig. 4 A, B presenting interchromatidian bridges in ana-telophase, Fig. 4 C, D, E presenting expelled chromosomes in metaphase, Fig. 4 F, exposing C-metaphase

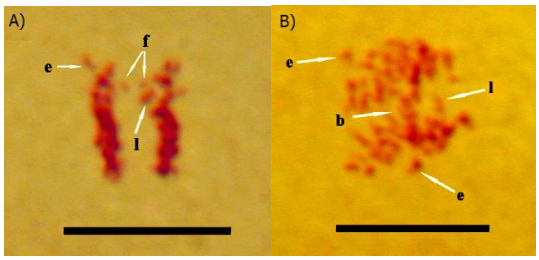


Fig. 5 – Abnormal dividing cells with complex chromosomal aberrations in meristemes of *Curcubita pepo* L. A) ana-telophase with lagged chromosome (l), together with expelled chromosome (e), and two chromosome fragments (f); B) metaphase with lagged chromosome (l) accompanied by two expelled chromosomes (e) and interchromatidian bridge (b); Bar = 10 μm.

Also images with complex aberrations (several aberrations together in the same cell) were captured such as: Fig. 5 A presenting abnormal ana-telophase with lagged (l) chromosome, expelled (e) chromosome and two chromosome fragments (f); Fig. 5 B presenting abnormal metaphase with interchromatidian bridge (b), lagged chromosome (l) and expelled chromosomes (e). The amplitude of UV-C genotoxic effect could be different for different plant species, as shown for maize, investigated by us earlier [21]; indeed, in that case we reported non-significant

influence on mitotic index but remarkable cytogenetic effects and also changes in photoassimilatory pigments levels. It worth to say that some authors [22] succeeded to evidence opposite effects of low levels UV irradiation (stimulatory effects) and higher levels of exposure (inhibitory and damaging effects).

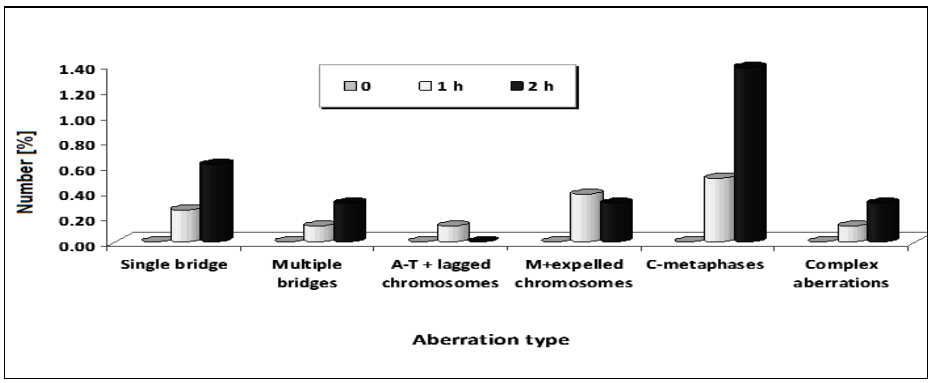


Fig. 6 – Chromosomal aberrations identified following UV exposure of root meristemes; M-metaphase; A-T - ana-telophase.

*Table 2*

Types of chromosomal aberrations induced in the UV exposed samples

| UV exposure time (h) | Normal dividing cells | Abnormal dividing cells | Clastogenic effect | |
|---|---|---|---|---|
| | | | Single bridge | Multiple bridges |
| 0 | 756 | 0 | 0 | 0 |
| 1 | 784 | 11 | 2 | 1 |
| 2 | 632 | 19 | 4 | 2 |
| UV exposure time (h) | Aneugenic effect | | | Complex aberrations |
| | Lagged chromosomes in ana-telophase | Expelled chromosomes in metaphase | C-metaphases | |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 3 | 4 | 1 |
| 2 | 0 | 2 | 9 | 2 |

According to Table 2 and Fig. 6, the frequency of C-metaphases and single interchromatidian bridges appeared to be remarkably increased in the UV

irradiated samples, thus it could be assumed that in the present experiment, with unique exposures of germinated seeds of pumpkin, the UV-C aneugenic action was best evidenced bioeffect. In the same time all the other described chromosomal aberrations were also identified in smaller number.

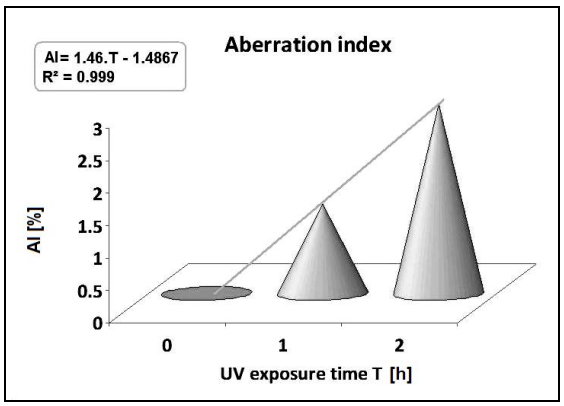


Fig. 7 – Chromosomal aberration percentage induced by UV exposure.

In Fig. 7 the aberration index is illustrated and dose-response linear curve was found, with correlation coefficient of 0.999.

We need underline two aspects still discussed in literature: (i) the types of chromosomal aberrations are not specific to the genotoxic agent [23]; (ii) not all cytogenetic changes identified in the meristemes persist in the vegetal organism developed from freshly germinated and irradiated seeds due to the cellular protective mechanisms, able to repair part of damages induced in early ontogenetic stages.

However, when certain chromosomal aberrations are transmitted to next generation cells they clearly could result in genetic mutations.

Considering the water content of irradiated vegetal samples as well as oxygen solubility in water, cell adaptation to the radiation effects should include the enhancing of antioxidative systems, activated supplementary to balance the reactivity of ROS species generated by photolysis and free radicals recombination.

Chromosome damages involve not only changes in the DNA molecules but also in the proteins from chromosome constitution since those also absorb preferentially optic radiation in the UV range. The influencing of redox processes by the induced ROS photoproducts could further perturb the integrity of cellular membrane system through lipid peroxidation. This is why, possible natural mutations in agroindustrial or pharmaceutical plants exposed to atmospheric UV rays require multidisciplinary scientific approach and full attention for environmental management. The specificity of genotoxic actions of UV-A, UV-B

and UV-C is still subject of research interpretation; we mention the reports of [20] that found the same types of cytogenetic changes as in our UV-C study (clastogenic and aneugenic changes) but induced by UV-B radiations.

Our results obtained in the present study on pumpkin are concordant with those reported for wheat [23] and for beans [16] that also evidenced the diminution of cell division ability [24-27]. As genetic investigation on plants are considered to provide valuable results for animal cell tissues also [28], plants are frequently taken as model organisms in cytogenetics, based also on their availability, not expensive costs and no ethical restrictions as in the case of animal biological material.

## 4. CONCLUSIONS

The exposure of freshly germinated pumpkin seeds to single UV-C irradiation of one hour and two hours respectively, have resulted in notable differences compared to control non-exposed biological material. Reduced number of cells in first mitosis phase, the prophase, was revealed, together with various increases of cells in the next division phases. Chromosomal aberrations found in the UV-C exposed samples were consistent with significant aneugenic radiation action: lagged and expelled chromosomes and C-metaphases and also with clastogenic action: single and multiple interchromatidian bridges. Linear dose-response graphs were revealed for mitotic index and chromosomal aberration index, with correlation coefficients of 0.999 in both cases. Further studies are going to be carried out for other exposure types, with either single or repeated irradiation with UV light.

## REFERENCES

1. J. F. Bornman, P. W. Barnes, S. A. Robinson, C. L. Ballaré, S. D. Flinte and M. M. Caldwellf, Solar ultraviolet radiation and ozone depletion driven climate change: effects on terrestrial ecosystems, Photochem. Photobiol. Sci., **14**, 88-107 (2015).
2. E. Stapleton, Ultraviolet radiation and plants: burning questions, Plant Cell, **4**, 1353-1358 (1992).
3. J. Rozema, J. Van de Staaij, L. O. Bjom, and M. Caldwell, UVB as an environmental factor in plant life: stress and regulation, Tree, **12**, 22-28 (1997).
4. D. Castronuovo, and G. Tataranni, UVC irradiation effects on young tomato plants: preliminary results, Pak. J. Bot., **46**, 945-949 (2014).
5. S. Zagan, B. Negreanu-Pîrjol, and T. Neg, Effect of ultraviolet radiations on the microbiological quality of drinkable groundwater, Environ. Eng. Manag. J., **10**, 1687-1692 (2011).
6. N. Lonigro, P. Montemurro, P. Rubino, P. Vergine, and A. Pollice, Reuse of treated municipal wastewater for irrigation in Apulia region: The "In.Te.R.R.A." Project, Environ. Eng. Manag. J., **14**, 1665-1674 (2015).
7. K. G. K. Cleminis, V. M Ranceliene, A. J. Prijalgauskiene, N. V. Tiunaitiene, A. M. Rudzianskaite, and Z. J. Jancys, Chromosome and DNA damage and their repair in higher plants irradiated with short-wave ultraviolet light, Mutat. Res., **181**, 9-16 (1987).

8. T. Hashimoto, C. Shichijo, and H. Yatsuhashl, Ultraviolet action spectra for the induction and inhibition of anthocyanin synthesis in broom sorghum seedlings, J. Photochem. Photobiol., **11**, 353-363 (1991).
9. M. Tevini, W. Iwanzik, and U. Thoma, Some effects of enhanced UV-B Irradiation on the growth and composition of plants, Planta, 153, 388-394 (1981).
10. J. L. Campos, X. Figueras, M. T. Pinol, A. Boronat, and A. F. Tiburcio, Carotenoid and conjugated polyamine levels as indicators of Ultraviolet-C induced stress in Arabidopsis thaliana, J. Photochem. Photobiol., **53**, 689-693 (1991).
11. R. P. Sinha, and D.-P. Hader, UV-induces DNA damage and repair: a review, Photochem. Photobiol. Sci., **1**, 225-236 (2002).
12. F. Thoma, Light and dark in chromatin repair: repair of UV induced DNA lesions by photolyase and nucleotide excision repair, EMBO J., **18**, 6585–6598 (1999).
13. F. Uehara, S. Miwa, Y. Tome, Y. Hiroshima, S. Yano, M. Yamamoto, E. Efimova, Y. Matsumoto, H. Maehara, M. Bouvet, F. Kanaya, and R. M. Hoffman, Comparison of UVB and UVC effects on the DNA damage-response protein 53BP1 in human pancreatic cancer, J. Cell. Biochem., **115**, 1724-1728 (2014).
14. C. Silva-Júnior, K. M. Asad, I. Felzenszwalb, and N. R. Asad, The role of Fpg protein in UVC-induced DNA lesions, Redox Rep., **17**, 95-100 (2012).
15. S.-C. Wang, H.-XJi., C.-L. Hsiao, T.-C. Wang, Y.-R. Syu, C.-P. Miao, K.-L. Hou, S.-S Lin., W.-S. Chang, and C.-W. Tsai, Protective effects of pyridoxamine against UVC-induced programmed cell death in HaCaT cells, In Vivo, **29**, 379-384 (2015).
16. I. C. Bara, Cytogenetic effects of irradiation with UV at six Romanian cultivars of Phaseolus vulgaris, An. St. Univ. Al. I. Cuza, Iasi, Romania, S. Genet. Biol. Molec., **X**, 51-55 (2009).
17. J. Armalyte, and K. Zukas., Evaluation of UVC-induced DNA damage by SCGE assay and its repair in barley, Biologija, **3**, 57-64 (2002).
18. E. Truta, D. Gherghel, I. C. Bara, and G. Vochita, Zinc-induced genotoxic effects in root meristems of barley seedlings, Notulae Botanicae Horti Agrobotanici, **41**, 150-156 (2013).
19. R. J. Singh, Plant Cytogenetics, Second Edition, CRC Press, Boca Raton, USA, 213, 2003.
20. A. Pandey, and G. Kumar, Clastogenic and aneugenic responses of supplemental UVB radiation on root meristems of lentil, Int. J. Life Sci. Res., **4**, 1-8 (2016).
21. C. Uta, A. Gritco Toderascu, G. Vochita, C. Nadejde, and D. Creanga, Spectral and microscopy study on UV-C radiation bioeffects in some vegetal organisms, J. Sci. Arts, **2**, 141-148 (2015).
22. F. Liu, H. Chen, and R. Han, Different doses of the enhanced UVB radiation effects on wheat somatic cell division, Cell Biol., **4**, 30-36 (2015).
23. E. A. Haliem, H. Abdullah, A. Asma, and A. L. Huqail, Oxidative damage and mutagenic potency of fast neutron and UVB radiation in pollen mother cells and seed yield of Vicia faba L., BioMed. Res. Int., art. 824656 (2013).
24. L. Hopkins, A. Hewitt, and U. Mark, Radiation reduces the rates of cell division and elongation in the primary leaf wheat, Plant Cell Environ., **25**, 617-624 (2002).
25. G. Kumar and A. Pandey, Effect of UV-B radiation on chromosomal organisation and biochemical constituents of *Coriandrum sativum* L., J. Biol. Sci., **10,** 85 – 93 (2017).
26. Y. Kara, Morphological and physiological effects of UV-C radiation on bean plant *(Phaseolus vulgaris*), Biosci. Res., **10**, 29-32 (2013).
27. D. Castronuovo, A. Sofo, S. Lovelli, V. Candido and A. Scopa, Effects of UV-C radiation on common dandelion and purple coneflower: First results, Int. J. Plant Biol., **8**, 61-64 (2017).
28. I. Kovalchuk, O. Kovalchuk, and B. Hohn, Biomonitoring the genotoxicity of environmental factors with transgenic plants, Trends Plant. Sci., **6**, 306-310 (2001).