RAD Conference Proceedings, vol. 4, pp. 39–44, 2020
ISSN 2466-4626 (online) | DOI: 10.21175/RadProc.2020.08
www.rad-proceedings.org

# INDUCTION OF MICRONUCLEI AFTER PROLONGED UV IRRADIATION OF POACEAE SPECIES CULTIVATED IN LABORATORY CONDITIONS AND WILD-GROWING IN RILA MOUNTAIN

**Tsveta Angelova[1*], Nikolai Tyutyundzhiev[2], Christo Angelov[3], Svetla Gateva[1], Gabriele Jovtchev[1]**

[1]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Central Laboratory of Solar Energy and New Energy Sources, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]BEO-Moussala, Institute for Nuclear Researches and Nuclear Energy, Bulgarian Academy of Sciences, Sofia, Bulgaria

***Abstract.*** *The aim of this study is to assess the potential of prolonged UV irradiation to induce genotoxic alteration in Poaceae species cultivated in laboratory and in mountain conditions. Changes in natural environment increase to a great extent with altitude. In natural ecosystems plants are exposed to UV and other environmental factors for more than one period of time of 10, 20, 30 or 43 days. Four wild species: Poa alpina L., Sesleria coerulans Friv., Festuca valida (R. Uechtr.) Pénzes, Dactylis glomerata L., characteristic of the ecosystems in Rila Mountain at three different altitudes (1500m, 1782m, and 2925m) were collected in three successive growing seasons (2017, 2018, 2019). Five-days old model plant Hordeum vulgare L. was cultivated and exposed to UV irradiation in laboratory conditions for periods of 10, 20, 30 and 43 days. Induction of micronuclei was applied as endpoint. We propose that: i) prolonged irradiation as well as its increase with altitude could induce higher genotoxic injuries in plants; ii) wild plants in mountainous and alpine biotopes are well adapted to the environmental conditions where a combination of abiotic stress factors can occur. Our results show variability in the response to UV irradiation between plant species cultivated in laboratory conditions and wild plants in natural environment where UV is combined with other abiotic stress factors. Micronuclei induced in H. vulgare in laboratory conditions were with higher frequency than those in plants growing in mountain conditions. It could be due to the fact that in laboratory conditions we studied the effect of a single factor and for a limited period of time, while in the natural environment the effect of prolonged UV irradiation is combined with other abiotic stress factors. Plant species at the highest altitude of 2925 m had a well pronounced low level of damage, despite expected high level of damage. It is well known that plants' response is modified when the effect of UV irradiation is combined with other factors. Further studies are needed for better understanding the mechanisms of interaction between factors and plant responses to the changing environmental conditions. Based on this and on future monitoring studies it could be possible to select sensitive monitor/model Poaceae species for the following comparative environmental impact assessments in laboratory and in mountain conditions.*

***Keywords:*** *Altitude,* Hordeum vulgare *L., micronuclei (MN), UV irradiation, wild plant species*

## 1. Introduction

One of the most important environmental factors that influence plants' growth, development and productivity is ultraviolet (UV) radiation. As part of solar electromagnetic radiation spectrum and based on its biological effects UV radiation is divided into: UV-C (100-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). UV-C with the shortest wavelength (100–280 nm) is extremely harmful for living organisms but not present in natural solar radiation at ground level, because it is completely absorbed by the ozone layer and atmospheric oxygen. While UV-A with the longest wavelength (315–400 nm) is slightly absorbed and directly submitted to the earth surface. The minor component of sunlight (1.5% of total radiation) is UV-B (280–315 nm) radiation which is filtered through the stratospheric ozone layer and only a small portion reaches the earth surface, but could induce a variety of damaging effects on all living organisms. In recent years, the impact of increased UV-B radiation on the biosphere, as a result of stratospheric ozone depletion, has heightened knowledge of the cytotoxic, mutagenic, and carcinogenic consequences of UV-irradiation, and also heightened the interest of the mechanisms of cell repair/tolerance to UV-induced DNA damage [1]. For plants this exposure is particularly harmful due to their binding requirement for sunlight to survive and their inability to move. UV-B could adversely react with amino acids, nucleic acids, lipids and could induce stress responses at molecular, cellular and organism levels [2]. Despite the very stable nature of plant genome, nuclear DNA is an inherently unstable molecule and could be damaged spontaneously, metabolically or by effect of stress factors. At molecular level UV-B damage to DNA could result to genotoxic stress, which could reduce plant genome stability, growth and productivity.

As sessile, photosynthetic organisms, without ability to move, plants have to cope with constant exposure of more than one environmental stress factor: increased ultraviolet (UV) irradiation, altitude, low and high temperature, humidity, desiccation, rehydration

* angelova_ts@abv.bg

and etc. It is well known that plant response differs when environmental factors act alone or in combination with other abiotic stress factors. Environmental factors change to a great extent with altitude. The amount of UV-B and UV-A reaching the ground varies with time of the day, day of the year depending on the weather, climate and geographical conditions, season of the year, altitude, latitude, atmospheric surface [3], [4]. The levels of UV-A and UV-B radiation and light intensity increase with elevation. It was found that for every 1000 m increase in altitude, the UV levels increase by 10 to 12% [5]. UV irradiance increases with altitude due to the shorter path length of the solar beam through the atmosphere and the consequent decrease of scattering and absorption [6]. As a result plants that naturally grow at higher altitudes are exposed to a higher extent both of UV-A and UV-B radiation. On the other hand, in mountain conditions except UV-A, UV-B irradiation and altitude there are many other abiotic factors such as local meteorological conditions, cloud cover, season, time of day, solar zenith angle, altitude, air pollution, etc. Therefore, in the areas of high altitude, like mountains, plants are exposed to more than one abiotic stress factor (they are under combined abiotic stress) [7]. Some authors found that in species growing in conditions with elevated background of UV-B radiation, such as in Alpine band and in the tropical latitudes, tolerance to the action of UV-B radiation grows with the increase of the ultraviolet level [8], [9]. Other authors reported that UV-B irradiation modifies the action of other ecological factors [10], [11], [12].

Higher plants have different sensitivity to solar UV radiation that depends on a complex of genetic characteristics, such as genotype, phenotype and ecotype [13]. Based on their sensitivity plants could be sensitive to UV-B radiation, with medium sensitivity or insensitive to UV-B radiation [14], [15]. It is known that dicotyledonous species are more sensitive to UV-B radiation than monocotyledonous species [16]. The objects of this study are one model and four wild species from family Poaceae which are supposed to be sensitive to UV-B radiation. This is a grass family of monocotyledonous flowering plants, a division of the order Poales, the most abundant and important family of the Earth's flora which grow on all continents, in desert, freshwater, marine habitats, and at all but the highest elevations [17].

It is known that it is not so much the absolute change in temperature, UV radiation and other environmental factors that are decisive for the survival/adaptation of the individual species, but the speed and direction of the changes. Based on this, here we hypothetically presume that prolonged irradiation as well as its increase with the altitude could induce higher genotoxic injuries in plants; or wild plants well adapt to the environmental conditions in mountainous and alpine biotopes. To check our hypothesis one model plant (widely used as a test object in cytogenetics) and four wild plant species from the same family Poaceae were used in different conditions. Even though these plant species are members of the same family they would have different ability to adapt.

The aim of this study is to assess the potential of prolonged UV irradiation both in laboratory and in mountain conditions to induce genotoxic alteration in cultivated and wild Poaceae plants.

## 2. Materials and Methods

### *2.1. Plant material*

Plant species from Poaceae family cultivated in different experimental conditions (one model and four wild plants) were studied.

### *2.2. Mountain conditions-characterization of the experimental sites:*

Four wild plant species were collected at three different experimental sites at different altitudes ("Skakavcite"-1500 m, "III window"-1782 m, Moussala-2925 m) in three successive growing seasons (2017, 2018, 2019). The selected plants are characteristic of the ecosystems in Rila Mountain for the respective altitude. The Poaceae species were collected in locations with similar environmental conditions (sunny, cloudy, shady or sheltered from the wind, etc.). So, one can assume that they were exposed to a comparable environmental impact in the respective biotopes.

The first experimental site in Rila Mountain is locality named "Skakavcite" above the village Beli Iskar (Bulgaria) at 1500 m a.s.l. (42° 12′ 08.52″ N; 23° 33′ 06.38″E). The soil type at this experimental site is brown forest with low clay content and high content of organic matter. Soil pH levels range between 4.5 and 6.0 [18]. *Dactylis glomerata* L. species of family Poaceae were collected around this site for three consecutive seasons.

The second experimental site is in the region of the locality named "Third window" at 1782 m a.s.l. (42° 10′ 27.70″ N; 23° 33′33.20″ E). This experimental site has dark mountain-forest soils with acidic pH ranging between 4.0 and 5.0 [18]. *D. glomerata* species were also collected around this site for three consecutive seasons.

The third experimental site is Moussala Peak (https://www.inrne.bas.bg/index.php/laboratories/basic-environmental-observatory-moussala). The plant diversity decreased rapidly with increasing altitude. The vegetation at Moussala Peak at 2925 m a.s.l. (42°10′ 45.13″ N; 23° 35′ 06.81″ E) and the surrounding area is of alpine type. It comprises a suite of azonal scree and rock vegetation, calcareous and acid grasslands and sedge heaths, snowbeds, soligenous mires, and spring vegetation. Above the tree line, the most important habitats are grasslands where the snow remains for more than 210 days. The species that grow here can survive in poor soil, lower temperature and short summer. The soil type in this experimental site is mountain-meadow alpine with pH values of approximately 4.5 [18]. Three wild Poaceae plants were collected in this site: *Poa alpina* L. (2017), *Sesleria coerulans* Friv. (2017) and *Festuca valida* (R. Uechtr.) Pénzes (2019).

### *2.3. Laboratory conditions-UV treatment*

A model plant, *H. vulgare* L. (spring barley; 2n = 14; standard karyotype) was used to assess the effect of UV-irradiation on plant DNA in laboratory conditions. Barley seeds were germinated and grown at standard conditions at room temperature. Five days old plants were irradiated in UV simulator for periods of 10, 20, 30 and 43 days. *H. vulgare* plants were cultivated in UV chamber prepared by N. Tyutyundzhiev (see Figure 1) [19], [20].

The aim of UV simulator experiments is to distinguish the effect of UV light on plants only (without PAR spectrum). The UV simulator is assembled as a ventilated container with an approximate area of 1 $m^2$ and a solar UV light simulator. The solar spectrum is simulated by a series of suitable artificial UV-A and UV-B light sources consist of narrow-band UV light sources – UV LEDs, UV LED strips and low-power UV fluorescent lamps (see Figure 1B).

- UV A light source consists of UV LED diodes with max emitting wavelength at 405 nm and total installed power of 15 W.

- UV-B spectrum is adjusted not by filtering but by adjustment of max intensity of UV-B lamp (central wavelength, 310 nm).

Due to variability of LED emissivity before any application we have regularly tested the emitting spectrum by portable multi-channel LED spectrometer.

Aluminum sheet is mounted on the internal wall of the container and its mirror surface is used as UV light diffuser and reflector.

The UV light dose (laboratory setup) is designed on the base of illumination time and instantaneous UV power during illumination. Thus, the real-time UV monitoring system has recorded typical irradiation value of 18 mW/$m^2$ per hour for UV A+UV B.

The daily UV dose is calculated experimentally following our on-field (40 days campaign) UV measurements at high-mountain research station in Rila Mountain (Moussala Peak-2925 m a.s.l.). The UV dose measured there (during summer campaigns 2017-2018, June-July) is accepted as the max daily UV dose which could be accumulated in our local climatic conditions. Occasional UV-C peaks are observed on the top of mountain. These findings could imply that tropospheric ozone is generated and the effect of additional ozone have to be taken into account. The same UV sensor system (UV-A, UV-B, UV-C) and the same daily UV dose is used to calibrate the above-mentioned UV simulator [19].

The UV exposure in the laboratory experiment was adapted to the real conditions on Moussala Peak (2925 m a.s.l.), namely 27.408 ± 0.348 Wh/$m^2$ versus 28.004 ± 0.654 Wh/$m^2$ for the entire duration of the experiment (43 days). The lower altitudes 1500 m a.s.l. and 1782 m a.s.l. shows a slightly lower exposure – around 24 Wh/$m^2$, which was not taken into account in the laboratory experiment.

### *2.4. Micronucleus assay*

Induction of micronuclei was used as a sensitive test for assessment of the genotoxic effect of UV radiation and other abiotic stress factors. Micronuclei were evaluated at 10, 20, 30 and 43 days after UV irradiation in laboratory conditions in barley leaves and in leaves of wild plants in the successive growing season in mountain conditions (2017, 2018, 2019) (see Figure 2). Unirradiated barley cultivated at laboratory conditions were used as a control.

For assessing the formation of micronuclei, the leaves were fixed in ethanol/glacial acetic acid (3:1) [5], [20], [21]. The micronucleus test was adapted and applied for leaves of *H. vulgare* [20] and for leaves of wild plants species [5].

Cells with at least one micronucleus per 1000 cells were analyzed. The results are presented as micronuclei (%) mean ± SD, where SD is the standard deviation. A total of 3000 cells for micronuclei per plant material and experimental area were evaluated for each group. The experiments were duplicated.

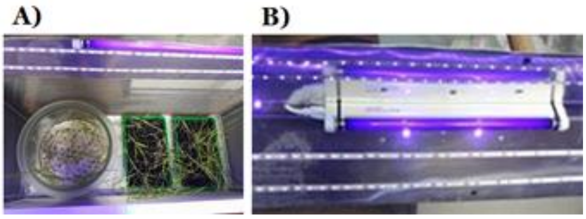


Figure 1. UV simulator: A) UV chamber with growing plants, B) Artificial UV-A and UV-B light sources.

### *2.5. Statistical data analysis*

Statistical analysis of micronucleus test was carried out using two-tailed Fisher's exact test for group comparison of different plant species [22], [5].

### 3. Results

The micronuclei formation was investigated on cultivated *H. vulgare* and wild Poaceae plants grown both in laboratory and in mountain conditions.

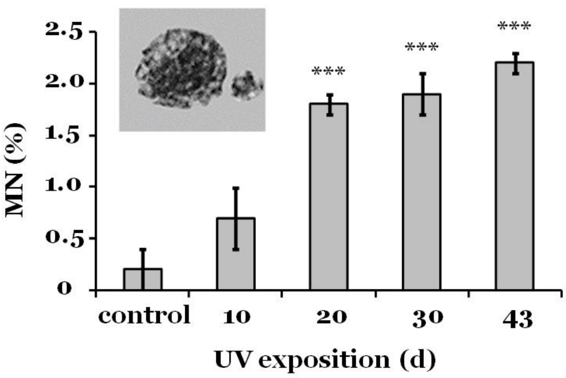


Figure 2. The exposure time - dependent formation of micronuclei in barley leaves. Statistical difference between leaves at different UV time duration exposure compared to the unirradiated control *** $p<0.001$.

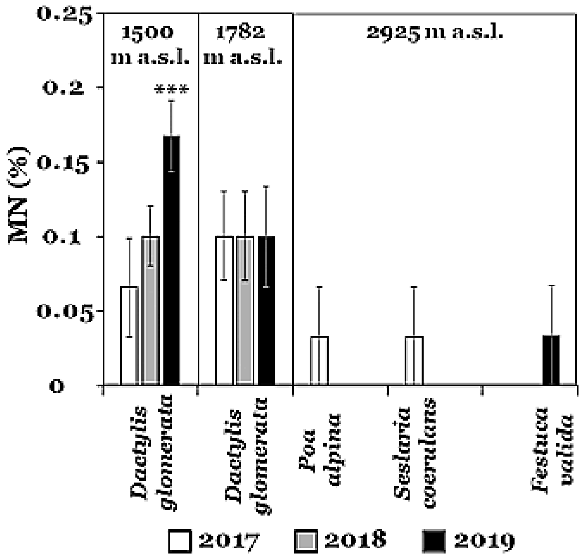


Figure 3. Micronuclei induced by UV radiation and other abiotic stress factors in leaves of wild plant species from three altitudes in Rila Mountain, Bulgaria. ***$p \leq 0.001$

The effect of prolonged UV irradiation was studied in laboratory conditions in barley in our previous work [20]. The frequency of induced micronuclei in barley leaves increased in a time-dependent manner (see Figure 2) (0.7% ± 0.3 after 10 days; 1.8% ± 0.1 after 20 days, $p<0.001$; 1.9% ± 0.2 after 30 days, $p<0.001$ and 2.2% ± 0.1 after 43 days of irradiation, $p<0.001$). After 20 days of irradiation the induced micronuclei had similar values.

Different effect of prolonged UV irradiation was obtained on wild Poaceae plants as frequency of micronlei formation in mountain conditions (see Figure 3). *D. glomerata* showed higher sensitivity among the four investigated wild Poaceae genotypes, in the growing seasons of 2017, 2018, 2019. The frequency of micronuclei was in the range of 0.033% ± 0.033 to 0.167% ± 0.024. The highest susceptibility of this genotype was measured for 2019 at 1500 m.a.s.l. ($p \leq 0.001$). Micronuclei for this wild plant grown at 1782 m a.s.l. showed close values for all studied seasons.

All Poaceae plants from Moussala Peak had relatively low frequency of micronuclei. *F. valida*, *P. alpina* and *S. coerulans* were not very sensitive in comparison with *D. glomerata*. The values of induced micronuclei were lower than that of *D. glomerata* (see Figure 3).

As can be seen from Figure 2 and Figure 3, induced micronuclei in *H. vulgare* irradiated in laboratory conditions were with higher frequency than that in wild plants growing in mountain conditions.

## 4. Discussion

Variability in the response to UV irradiation between cultivated species in laboratory conditions and wild plants in natural environment where UV is combined with other abiotic stress factors was found. Higher frequency of micronuclei was observed in plant irradiated in laboratory conditions than those in mountain conditions. The prolonged irradiation (43 days) was more genotoxic for leaf samples than the shorter ones (30, 20, 10 days). Our results are in agreement with [2], where it was found that the higher doses were more genotoxic than the lower ones and induce more aberrations in treated sets than the control. This is probably due to the accumulation of damage of DNA of plant cells that has not been repaired during the study period.

In our previous work with the same model plant *H. vulgare*, grown and irradiated at the same controlled conditions has found rapid plant adaptation assessed by molecular parameter comet assay [23]. An initial increase in DNA damage after 10 days of UV exposure was observed and the value of DNA migration (DNA in tail) after 20 days of irradiation was close to that of the untreated plants. On the other hand higher value of DNA damage was observed in wild plant species belonging to various families grown in mountain conditions in comparison with the cultivated plant. Based on this work it could be suggested that the higher values of DNA damage in wild plants could be due to the combined effect of UV irradiation and other abiotic factors in mountain conditions. Plant response is modified as the effect of UV radiation is combined with other abiotic stress factors. The role of the genotype is essential for the degree of adaptation and the plant response.

In our study we obtained different susceptibility for wild Poaceae species depending on the altitude. Despite of the fact that higher UV intensity on Moussala Peak suggests a higher frequency of micronuclei, in the present study wild species *F. valida, P. alpina* and *S. coerulans* we found lower level of damage at the highest altitude. Probably this could be due to some adaptations developed by the plants to overcome the UV-radiation and other abiotic stresses [5], [20] at high altitude.

Specific organ susceptibility to prolonged UV-irradiation was found in laboratory conditions that could be related to their different vital function [20]. In addition, difference in the sensitivity to prolonged UV irradiation was observed. That difference depends on: studied conditions, species from one family and plant organs in one genotype [5], [20], [23]. As mentioned above, as monocotyledonous species, family Poaceae is supposed to be sensitive to UV-B radiation, but to a lower extent than the dicotyledonous species. For more representative results of genotoxic effect of prolonged UV irradiation, more studies with more than one endpoint in different plant species are needed.

Although different species have been studied in laboratory experiments and in natural environment in mountain conditions, it can be assumed that they can react in a similar way to UV exposure. The wild plant species *D. glomerata*, *P. alpina*, *F. valida* and *S. coelurans* show similar results among themselves compared to *H. vulgare*. Hence, we can conclude that these plants have adapted to the higher UV exposure associated with the higher altitude combined with other abiotic factors. As can also be seen from the laboratory experiment, an adaptation to the UV radiation occurred. After just 20 days, the initially steep increase in induced micronuclei almost turned into a "straight line". For a real adaptation, of course, a much longer time is required, which is passed on and consolidated over generations.

So far we have examined only cytogenetic endpoints. In the ongoing project we are going to carry out biochemical investigations, such as total chlorophyll content, chlorophyll *a* and chlorophyll *b* content, as a result of the different photosynthetically active radiation (PAR). This will give a more complete picture of the effects.

## 5. Conclusion

The four wild plant species examined show a good adaptation to the environmental conditions (UV radiation and other abiotic factors) in the three biotopes in different altitudes in Rila Mountains. A low micronuclei induction was noted.

A significantly higher number of micronuclei was found in the laboratory experiment with another representative of the Poaceae family – barley. After 20 days of exposure to UV-A and UV-B, there was a visible adjustment. However, this adjustment takes place at a lower level.

The obtained by us findings showed that plant response is modified as the effect of UV radiation is combined with other abiotic stress factors. For better understanding the mechanisms of interaction between abiotic factors and plant response and adaptation to the changing environmental conditions, further studies with more than one endpoint are needed.

Based on this data as well as on future monitoring studies in other plants it could be possible to select the sensitive monitor Poaceae species for the comparative environmental impact assessments.

***Acknowledgements:*** *The paper is part the research done within the project under contract grant number DN 04/1 entitled: "Study of the combined effects of the natural radioactive background, UV radiation, climate change and cosmic rays on model groups of plant and animal organisms in mountain ecosystems.", supported by the Bulgarian Ministry of Education, Youth and Science.*

## References

1. S. W. Mpoloka, “Effects of prolonged UV-B exposure in plants“, *African Journal of Biotechnology*, vol. 7, no. 25, pp. 4874-4883, 2008.
Retrieved from:
http://www.academicjournals.org/AJB
2. G. Kumar, A. Pandey, “Effect of UV-B radiation on chromosomal organisation and biochemical constituents of *Coriandrum sativum* L.”, *Jordan Journal of Biological Sciences,* vol. 10, no. 2, pp. 85-93, 2017.
3. A. G. Roro, M. T. Terfa, K.A. Solhaug, A. Tsegaye, E. Olsen, S. Torre, “The impact of UV radiation at high altitudes close to the equator on morphology and productivity of pea (*Pisum sativum*) in different season”, *South African Journal of Botany*, vol. 106, pp. 119–128, 2016.
http://dx.doi.org/10.1016/j.sajb.2016.05.011
4. L. F. Suárez Salas, J. L. Flores Rojas, A. J. Pereira Filho, H. A. Karam, “Ultraviolet solar radiation in the tropical central Andes (12.0°S)”, *Photochem. Photobiol. Sci.*, vol. 16, pp. 954–971, 2017.
https://doi.org/10.1039/C6PP00161K
5. G. Jovtchev, A. Stankov, I. Ravnachka, S. Gateva, D. Dimitrov, N. Tyutyundzhiev, N. Nikolova, Ch. Angelov, “How can the natural radiation background affect DNA integrity in angiosperm plant species at different altitudes in Rila Mountain (Southwest Bulgaria)?”, *Environmental Science and Pollution Research*, vol. 26, pp. 13592–13601, 2019.
https://doi.org/10.1007/s11356-019-04872-1
6. Y. Sola, J. Lorente, E. Campmany, X. de Cabo, J. Bech, A. Redano, J. A. Martinez-Lozano, M. P. Utrillas, L. Alados-Arboledas, F. J. Olmo, J. P. Diaz, F. J. Exposito, V. Cachorro, M. Sorribas, A. Labajo, J. M. Vilaplana, A. M. Silva, J. Badosa, “Altitude effect in UV radiation during the evaluation of the effects of elevation and aerosols on the ultraviolet radiation 2002 (VELETA-2002) field campaign”. *J Geophys Res*, vol. 113: D23202, 2008.
https://doi.org/10.1029/2007JD009742
7. C. Körner, “The use of ‘altitude’ in ecological research”, *Trends Ecol. Evol.*, vol. 22, no. 11, pp. 569-574, 2007.
https://doi.org/10.1016/j.tree.2007.09.006
8. O. Aknazarov, “Effect of ultraviolet radiation on the growth, morphogenesis, and hormone level of alpine plants”, *Extended Abstract of Dr. Sci. (Biol.) Dissertation, Dushanbe*, 1991.
9. M. M. Caldwell, “Solar ultraviolet radiation as an ecological factor for alpine plants”, *Ecol. Monographs*, vol. 38, pp. 243–268, 1968.
https://doi.org/10.2307/1942430
10. M. M. Caldwell, C. L. Ballare, J. F. Bornman, S. D. Flint, L. O. Bjorn, A. H. Teramura, G. Kulandaivelu, M. Tevini, “Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors”, *Photochem. Photobiol. Sci.*, vol. 2, no. 1, pp. 29–38, 2003.
https://doi.org/10.1039/B211159B
11. J. H. Sullivan, A. H. Teramura, “Field study of the interaction between solar ultraviolet-B radiation and drought on photosynthesis and growth in soybean”, *Plant Physiol.*, vol. 92, no. 1, pp. 141–146, 1990.
12. S. Koti, K. R. Reddy, V. R. Reddy, V. G. Kakani, D. Zhou, “Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max*) flower and pollen morphology, production, germination and tube lengths”, *J. Exp. Bot.*, vol. 56, no. 412, pp. 725–736, 2004.
https://doi.org/10.1016/j.envpol.2005.01.030
13. Á. Ferrero-Serrano, S.M. Assmann, “Phenotypic and genome-wide association with the local environment of Arabidopsis”, Nat. Ecol. Evol. vol. 3, 274–285, 2019.
https://doi.org/10.1038/s41559-018-0754-5
14. E.V. Kanash, Influence of UV-B Radiation on Agroeconomic Systems, *Dokl. Vseross. Akad. Sel’skokhoz. Nauk,* vol. 3, pp. 17–20, 2002.
Retrieved from:
https://scholar.google.com/citations?user=JJ1nLq8AAAAJ&hl=en&oi=sra
15. B.R. Jordan. The Effect of Ultraviolet-B Radiation on Plants: *A Molecular Perspective, Adv. Bot. Res.*, vol. 122, pp. 97–162, 1996.
Retrieved from:
https://scholar.google.com/scholar_lookup?title=The%20Effect%20of%20Ultraviolet-B%20Radiation%20on%20Plants%3A%20A%20Molecular%20Perspective&journal=Adv.%20Bot.%20Res.&volume=122&pages=97-162&publication_year=1996&author=Jordan%2CB.R

16. R. Rai, S. Singh, S. Yadav, A. Chatterjee, S. Rai, A. Shankar, L. C. Rai, "Impact of UV-B radiation on photosynthesis and productivity of crop. Environment and photosynthesis a future prospect" in *V. P. Singh, S. Singh, R. Singh, P. K. Srivastava, S. M. Prasad,* Eds., Environment and photosynthesis: a future prospect. Studium Press, New Delhi, 2018, pp. 336–346.
17. C.S. Campbell, Poaceae, Website Name: Encyclopaedia Britannica, Publisher: Encyclopaedia Britannica, Inc., Date Published: 07 October 2016. Retrieved from: https://www.britannica.com/plant/Poaceae
18. Rila National Park, Management Plan 2001–2010, published 2001, p. 289. Retrieved from: http://rilanationalpark.bg/assets/userfiles/Rila%20NP-en.pdf
19. N. Tyutyundzhiev, Ch. Angelov, K. Lovchino, Hr. Nitchev, M. Petrov, T. Arsov, "Modeling the natural UV irradiation and comparative UV measurements at Moussala BEO (BG)". *IOP Publishing, J Phys: Conf. Ser.* 992 (012022): pp. 1–7, 2018. https://doi.org/10.1088/1742-6596/992/1/012022
20. Ts. Angelova, A. Stankov, N. Tyutyundzhiev, Ch. Ivanov, S. Gateva, G. Jovtchev, "Does prolonged UV irradiation induce genotoxic effect on *Hordeum vulgare* L.?," in *23rd International Eco-conference of environmental protection of urban and suburban settlements, Proc., 25th-27th September, Novi Sad, Serbia*, 2019, pp. 137-143, ISBN 978-86-83177-55-4.
21. G. Jovtchev, M. Stergios, I. Schubert, "A comparison of N-methyl-N-nitrosourea-induced chromatid aberrations and micronuclei in barley meristems using FISH techniques", *Mutation Research*, vol. 517, pp. 47–51, 2002. https://doi.org/10.1016/s1383-5718(02)00038-4
22. G. Jovtchev, M. Menke, I. Schubert, "The comet assay detects adaptation to MNU-induced DNA damage in barley", *Mutation Research*, vol. 493, pp. 95–100, 2001. https://doi.org/10.1016/S1383-5718(01)00166-8
23. G. Jovtchev, S. Gateva, Ts. Angelova, K. Katrandzhiev, N. Nikolova, D. Dimitrov, Ch. Angelov, "Impact of UV radiation on the DNA of plants at different altitudes in Rila Mountain, Bulgaria-a three years study", in *24 International Eco-conference, 11 Safe Food, Proc., 23–25th September, Novi Sad, Serbia,* 2020, pp. 59-67. ISBN 978-86-83117-56-1.