JOURNAL OF
EMERGING INVESTIGATORS

Article

The non-thermal effect of UV-B irradiation on onion growth

Mohamed Nashnoush, Judy Rodgers
Yarmouth Consolidated Memorial High School, Yarmouth, Nova Scotia

SUMMARY

Both terrestrial and aquatic plants, the chief autotrophs supporting life on earth, can be threatened by global warming and particularly by UV-B radiation due to the depletion of the ozone layer. Ozone depletion may also threaten the biodiversity of ecosystems and dismantle food webs. The deleterious effects of UV-B have been studied mostly through *in vitro* studies and vary significantly according to the dose received, the irradiation period and the sensitivity of the species. Examined adaptive mechanisms encompass increases in antioxidant enzymes, phenolic compounds and flavonoids which function as protective screens. The interaction of UV-B radiation with DNA, lipid and protein molecules is vital in determining how photosynthesis and cellular respiration are affected by UV-B radiation. Hence, this study seeks to explore the non-thermal effects of UV-B irradiation on the physiology and morphology of *Allium cepa*. This was completed by comparing the mitotic index of the control to the irradiated populations. A paired samples two-tailed t-test was performed, and the results demonstrated a decline in mitotic vitality, suggesting that UV-B can generate biochemical stress, which can influence *Allium cepa*'s physiology.

INTRODUCTION

The Montreal Protocol banned the use of chlorofluorocarbons (CFCs) due to ozone layer depletion and increased exposure to harmful short wavelength radiation (1). CFCs disrupt the ozone-oxygen cycle by reacting with radical oxygen atoms, preventing the formation of ozone (1). The "ozone hole" is predicted to be twenty-four million kilometers in diameter, allowing ultraviolet (UV) radiation to penetrate through the troposphere, increasing the exposure of plants and mammals to ionizing radiation (1). There are now plenty of alternatives to the use of volatile CFCs; however, the struggle to completely eliminate this compound continues, as another source has been detected in the region of East Asia (1). Additionally, other volatile substances (e.g. sulphur oxides, nitrogen oxides, methane and organohalogens) have been found to deplete ozone levels (2). This calls on the need to explore the possible ramifications resulting from UV-B rays penetrating the stratosphere and interacting with crops (2). Onions' (*A. cepa*) indispensable dietary role and ubiquitous nature inspired their use in this study. Our research seeks to explore how the non-thermal effects of UV-B radiation at 300nm influence the growth of *Allium cepa* after zero, one, two, three, four and five hours of exposure.

There are three different types of UV radiation: UV-C, UV-B, and UV-A. UV-C possesses the highest amount of energy, with a wavelength between 100nm and 290nm (2, 3). This type of UV radiation does not penetrate the ozone layer and, consequently, does not reach the earth's surface (3). On the other hand, only a few UV-B rays with a wavelength between 280nm and 320nm penetrate the ozone layer (3). However, with the gradual ozone depletion at the stratosphere, increasing amounts of UV-B rays are entering (1, 2, 3). UV-A with wavelengths between 320nm and 400nm is considered benign relative to the aforementioned types of UV (3). UV-A mostly penetrates the ozone layer; however, the low amount of energy possessed by UV-A is not sufficient to pose any hazards to plants (3). UV-B was selected for examination in this study, as it is pertinent to both the Permian extinction and contemporary ozone depletion resulting from atmospheric pollutants (3). Further, an average wavelength of 300nm was adopted in the methodology to represent the wavelength segment of UV-B radiation fairly (4).

Work by Reboredo noted that, as a consequence of UV-B ambient irradiation, concentrations of chlorophyll a and chlorophyll b progressively declined (4, 5). Further, pigment degradation increased, and changes in the structure of Rubisco led to a loss in function (6). Thus, levels of photosynthesis are likely to decline, and concentrations of glucose will consequently decrease (6). This will lower the rate of respiration and lead to a reduction in the levels of ATP (6, 7). Reduction in the levels of ATP could lead to a variety of different events. One of the anticipated events would be cells entering G_0 phase, as ATP concentrations are not sufficient to allow Cyclin-dependent kinases to activate all enzymes required to perform roles for a specific stage of the cell cycle (8). Hence, it was hypothesized that the mitotic index would decrease as irradiation time increases.

Our results may be of global significance and could prompt the prevention of volatile substances that may threaten the biodiversity of ecosystems. Comprehending how plants respond to UV-B can allow us to gain a better grasp of the dietary risks associated with the consumption of irradiated varieties and the consequential effects on ecosystems.

RESULTS

To test the hypothesis the onion root tips were grown over a four-week period, where regular hydration, aeration

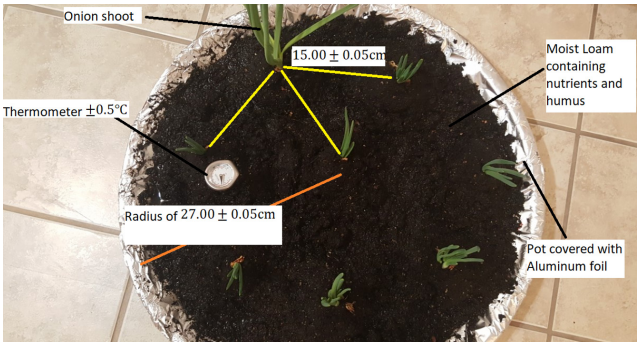

Figure 1: Experimental set up for growing onion sets. Figure highlights how all onion bulbs are equally spaced to reduce the prospect of intra-specific competition.

and sunlight exposure were conducted **(Figure 1)**. Some *A. cepa* varieties were subject to UV-B irradiation, and a control group was left untreated **(Figure 2)**. Root tips were carefully harvested and were utilized as a marker of cellular proliferation due to the presence of the apical meristem. Requisite raw data was collected to calculate mitotic indices of the irradiated and control groups.

After several weeks, most onion bulbs successfully sprouted. There was a noticeable increase in biomass and an increase in the girth of the shoots. Fortunately, saprotrophic mold did not develop, signaling adequate growth conditions (9). However, after the duration of four weeks, five onion bulbs failed to develop noticeable shoots. This is likely due to the varying ages of onion bulbs, which determines their ability to grow further. This also explains why some onions developed extensive shoots in a few days. Onion root cells within a forty-cell area were noted **(Figure 3)**. Lastly, it was noted after five hours of irradiation, the shoots appeared to sag, suggesting loss of cellular turgidity (9).

The high coefficient of determination (R^2) suggests the data points closely fit the regression line, which indicates that the line of best fit is a reliable illustration of the association between the variables **(Figure 4)**. Additionally, the negative linear trend observed is confirmed by the negative gradient of the equation of the line of best fit (y=-4.5x+49). To examine the extent of statistical dependence between the dependent and independent variables, the correlation coefficient (r) was calculated. The correlation coefficient was found to be r=-0.94, which suggests there is a strong negative correlation between the variables. Further, there is some overlap between the error bars, which alludes to trivial or non-existing differences between some sample means. Limitations certainly arise from high biological variance, which is indicated by the high standard deviation and standard error values across all samples, as seen in **Table 1.**

To deduce the extent of statistical significance in the data collected, we performed an inferential statistical test as illustrated in **Table 2**. The assumptions made by the paired samples two-tailed t-test were all satisfied by the data collected, which encouraged us to perform this particular statistical test. These assumptions included normality, continuous nature of the dependent variable, dependence of observations and random sampling, all of which have been satisfied, qualifying the data for this statistical test (10). The t-test was completed to compare two sample means from the same population, indicating if there is a significant statistical difference between the two samples. The results conclusively reveal that only the four- and five-hour groups are statically significant at 95% confidence. This is alluded to and foreshadowed by the substantial difference in the mean mitotic indices of both groups relative to the control. Such reduction in the mitotic vitality is investigated deeper in the discussion section.

DISCUSSION

As seen in the results section, the mean mitotic index decreases as irradiation time increases. This negative trend between the variables is visually represented by the line of best fit in **Figure 4**, which was discovered to be a reliable illustration of the relationship between variables due to the high coefficient of determination (R^2=0.89). Further, the negative correlation between the variables is deemed strong by the low correlation coefficient (r=-0.94). These results support the aforementioned hypothesis, and hence one can infer that as UV-B irradiation increases, the growth of onions decreases.

The decline in the mean mitotic index is largely due to the harmful effects of UV-B irradiation on the various leaf structures. Namely, UV-B radiation can reduce stomatal conductance by increasing concentrations of Abscisic acid

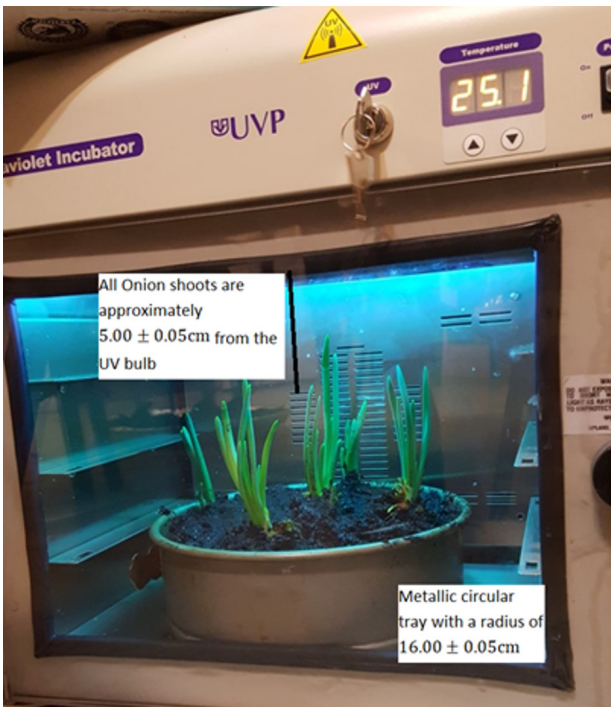

Figure 2: Experimental set up for irradiation of onions. The irradiation setup reveals how all onion shoots receive the same intensity of UV-B rays.

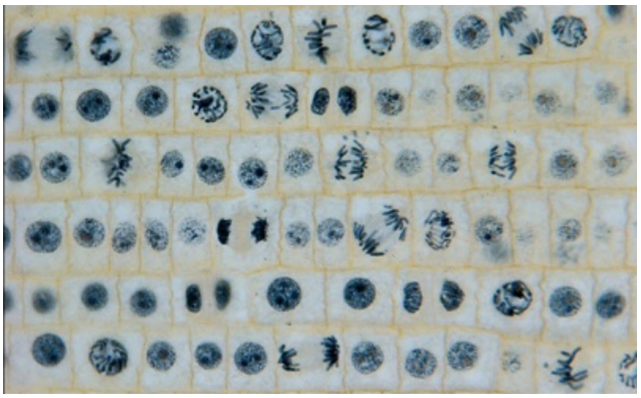

Figure 3: Micrograph of onion root tip. The micrograph of an onion root tip displays a 40-cell parameter, which was achieved at ×40 total magnification.

in the leaves (11). Concentrations of Abscisic acid would accumulate in the leaves due to biochemical stress (6, 9, 11). This could explain why the shoots appeared flaccid after irradiation, as the transpiration rate reached a halt, and negative pressure was no longer generated to mobilize water up the Xylem (11). Further, UV-B irradiation can generate oxyradicals, as oxygen molecules absorb higher amounts of energy carried by UV-B photons forming energetic radicals (6, 7, 11). These oxyradicals substantially inhibit the normal function of growth hormones, such as cytokinins and auxin (11, 12). This is because plant hormones are highly sensitive to photooxidative stress. Additionally, an increase in the activity of enzymes such as cytokinins dehydrogenase that would otherwise be regulated, uncontrollably inactivate cytokinins (12). Such effects would decrease the metabolic rate, as growth hormones would be present in small concentrations. Consequently, the rate of cellular proliferation in the roots would decrease and hence cells would likely enter the quiescent stage of G_0, or in extreme cases, go into cellular senescence (3, 6, 12). This could explain why a reduced number of mitotic cells were observed under the compound light microscope. Future iterations of this experiment should consider the use of flow cytometry techniques to objectively analyze the chemical constituent changes that accompany biochemical stress.

Our research could provide a deeper insight into one of the postulated causes of the Permian extinction, as mass extinction could be due to the increasing irradiation to UV-B and UV-C (1, 2, 12). Geologists and volcanologists have found that during the time of the Permian extinction, volcanoes were highly active, frequently erupting and releasing atmospheric pollutants, which could have thinned or debilitated the ozone layer (2, 11, 13). This would have increased UV exposure levels, and consequently, plants experienced biological stress, which likely led to the collapse of food webs due to a bottom-up factor (1, 2, 13). Populations essentially went extinct because of the dramatically low carrying capacity of the environment. Our study could contribute by outlining the change in the growth patterns accompanying irradiation, which would provide an indication of what likely happened to various crops present at the time of the Permian extinction.

However, it is important to note that the implications of our research are limited primarily due to our inability to reproduce the natural conditions of the environment. Additionally, limitations arise from high biological variance, which is indicated by the high standard deviation and standard error values across all samples. Due to differences in the genotype, it is very likely that some onions were more acclimated than others to UV-B radiation, which would have led to higher mitotic indices. This is a contributing factor to the high variability seen in the results. Further, as noted qualitatively, the onions had varying abilities to grow, suggesting onions were of different ages, and some may have already reached maturity.

Additionally, the absence of a drainage system and addition of a high volume of distilled water over the duration of four weeks limited our experimental set-up. The effect on onion bulbs is debatable because the container had a high depth. When transplanting, we realized onion bulbs had a tendency to develop lateral roots, as opposed to deep fibrous roots. Thus, it is implausible that aeration of the onions' shallow root network was reduced, as excess water buildup was recorded to occur at a depth of 10cm (i.e. far below the roots). However, adding a high volume of water may have led to leaching of valuable minerals and consequently reduced the potential for growth. This systematic error would reduce the mitotic index across all onions. However, it would not affect the conclusions reached regarding UV-B irradiation as onion samples are compared relative to one another.

It is important to note only 5mL of water was added per day to each onion bulb. This suggests that minimal evaporation occurred and over the span of four weeks; most of the water simply accumulated at the bed of the pots. One solution would be to increase temperatures (e.g. 30°) to evaporate excess water. Another solution is to drill holes in the pots, which will serve as a pathway to enable excess amounts of water to drain out. Further, a saucer can be added underneath to collect excessive amounts of water. The water collected by the saucer can be reused to water the onions, as the water will contain high quantities of dissolved minerals, which through mass flow of water can diffuse passively into the epidermal

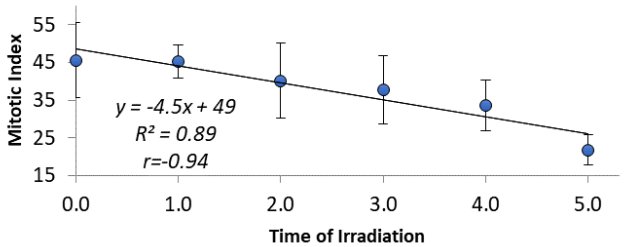

Figure 4: Mean mitotic indices at varying exposure times. Graphical representation of processed data accentuates the negative association between the variables. Note the vertical error bars were constructed using standard deviation to demonstrate the variability within the data..

Exposure Time	Mitotic indices (%)										
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Mean	Standard Deviation	Standard Error
0.0	36.36	55.17	50.00	42.86	26.53	47.37	46.34	60.53	45.7	9.96	3.8
1.0	53.57	39.47	46.67	41.18	41.03	47.22	44.74	48.65	45.3	4.40	1.7
2.0	26.47	37.50	27.91	37.93	38.98	43.75	51.72	57.14	40.2	9.91	3.7
3.0	44.19	34.38	28.21	23.26	35.29	40.00	42.86	53.85	37.8	9.02	3.4
4.0	39.29	29.41	26.32	25.00	30.30	39.29	33.33	45.83	33.6	6.80	2.6
5.0	21.88	28.57	17.95	16.22	22.45	21.88	26.67	18.75	21.8	3.96	1.5

Table 1: Processed data indicates a consistent decline in the mean mitotic index as the exposure period increases.

cells of the roots. This will reduce the need for the roots to actively pump in various nutrients into the roots and hence direct more of ATP reserves to fulfill growth requirements.

Another limitation is that plants were almost exclusively irradiated with UV-B and consequently, they were deprived of photosynthetically active radiation (PAR). An artificial source of UV-B does not match the diverse solar spectrum of the sun. Several studies have found that the effects of UV-B on vegetative growth and photosynthesis are mitigated by PAR and UV-A radiation (13, 14). For example, it has been found that visible blue light can increase the acclimation of plants by reducing the degradation of photosynthetic pigments (e.g. Chlorophyll a & b and Xanthophylls) and contributing to an increase in the content of protective oils on the epidermis layer (14). This can be rectified by installing a lamp that closely mimics solar radiation, emitting UV-B and PAR rays (e.g. Eye Hortilux PowerVEG T5 H0 Fluorescent Lamp Full Spectrum +UV).

All of these limitations would have led to high standard deviation values, limiting the reliability of the results. However, the standard deviation would be accounted for by the t-test. As seen in **Table 2**, the only two samples shown to be statistically different relative to the control are the 4-hour and 5-hour samples. This suggests that the damage induced by UV-B is not instantaneous and substantiates the mentioned hypothesis. Our findings align with various studies and more importantly, the research provides a supported answer to the research question despite its possible limitations.

Our research does not claim generalizability especially because some native species in high altitudes in the tropics and subtropics have evolved a UV-B sensing protein (UVR8) capable of expressing certain genes, which enable the plant to release various coating oils serving to protect the plant's leaves (14).

Field-based studies should be conducted to better represent the natural conditions of habitats, which can provide a more realistic indication of the consequences accompanying UV-B irradiation on crops. These field studies should strive to provide a comprehensive picture by considering other parameters (e.g. CO_2 concentration, fluctuations in temperatures, wind speed & topography). Additionally, further research should examine species at high altitudes with adaptive mechanisms, since this can reveal sensitive metabolic pathways. Compounds such as flavonoids, phenolics and anthocyanins, that are released during periods of biochemical stress can be used as biomarkers to detect sensitive areas of a plant (14, 15). Further, more research should be directed towards the regulation of UV-B irradiation at levels where physiological stress is minimized — namely, where irradiation does not present significant growth constraints. This is mainly because UV-B irradiation can increase the synthesis of antioxidants and protective oils, which can increase the medicinal content of crops (14, 15, 16). Additionally, regulated irradiation to UV-B can improve the flavor of some crops and increase the pungency of plant aromas, which may bring benefits to both the food and

		t-statistic	Degrees of Freedom	p-value
Pair 1	Control - 1hour sample	.082	7	.937
Pair 2	Control - 2hour sample	1.371	7	.213
Pair 3	Control - 3hour sample	1.829	7	.110
Pair 4	Control - 4hour sample	3.083	7	.018
Pair 5	Control - 5hour sample	5.939	7	.001

Table 2: The statistical results indicate that only variable pair four and five are statistically significant at 95% confidence.

fragrance industries (16). Lastly, different methods should be implemented to measure growth (e.g. dry mass measured by an electronic balance, rate of water absorption recorded by a potometer and use of chromatography techniques to evaluate pigment concentrations). Researchers should also consider elongating irradiation times without completely depriving plants of PAR. This would greatly contribute to our understanding of the effects induced by UV-B irradiation on *Allium cepa.*

MATERIALS AND METHODS

Growing Method

Seven pots with a diameter of 54.0cm and a depth of 11.0cm were first covered using 0.360m^2 of aluminum foil. Aluminum foil was used to help raise temperatures, as warmth stimulates secretions of various growth hormones (e.g. Auxin) that induce biochemical changes and prompt onions to exit their state of dormancy. Then, loam was added to each pot up to a height of 9.00cm. Eight onions were planted in each pot. When planting, all the tunicated bulbs were buried in the loam such that only the developing shoots project outwards **(Figure 1)**. Further, surrounding each bulb, 7.00g of NPK fertilizer was added. A thermometer was periodically placed into the loam to ensure maintenance of the temperature at 20.0°. Additionally, the humidity of the room was noted every day. Five milliliters of distilled water was added on a daily basis to the loam surrounding each onion shoot. Further, all pots were positioned in close proximity to a transparent window to allow equivalent amounts of sunlight to reach all onion bulbs. Onions were grown for a duration of four weeks to ensure onions had sufficient time to develop roots.

Irradiation and Microscopy Technique

After four weeks, most onions developed apparent shoots, which signaled the development of roots. Five mature onions bulbs were transplanted in a circular tray. The tray already contained loam, which allowed onions to be positioned as if in the natural environment. A ruler was used to ensure the distance between the onion shoots and UV-B light bulb was approximately 5.00cm **(Figure 2)**. Afterwards, the tray was placed in the UVP UV Incubator SI-950, and the incubator door was locked firmly. The temperature was set at 25.0°. Once UV-B irradiation began, the stopwatch was promptly started. After one hour, UV-B irradiation was ceased for a brief moment, and the incubator was opened to carefully remove a single onion bulb, which was then placed on the counter. This was done in one-hour intervals to obtain one-, two-, three-, four-, and five-hour irradiated onions. The maximum irradiation period was limited to only five hours, as a safety hazard is posed by surpassing the five-hour mark (17).

Once an onion bulb was irradiated for the designated period, a metal scalpel was used to carefully cut a segment 1.25cm long from the root tip. A glass rod was then rolled along the length of the root tip to forcefully squash the root tip. Then, 2.00mL of 0.1% Sigma-Aldrich Methylene blue was dispensed into an Eppendorf tube. The crushed length of the root was then placed in the Eppendorf tube, and the solution was vigorously swirled. After swirling, tweezers were then used to transfer the root tip onto a glass microscope slide. Then, 1.00mL of 0.1% Methylene blue was added to the microscope slide. Subsequently, a microscope coverslip was gently lowered at an angle on the onion root to avoid trapping air bubbles under the coverslip. The sample was then analyzed under a compound light microscope at ×40 total magnification. After the irradiated samples were viewed under the microscope, the control (0 hours) was promptly examined under the microscope. The numbers of mitotic and non-mitotic cells were noted for each sample. Forty-eight samples were examined under a compound light microscope to attain eight trials.

The different stages of the cell cycle were discerned by studying the changes in internal cellular structure accompanying phase transitions. The most apparent indication that cells are undergoing interphase is that the sister chromatids or chromosomes are not visible under a compound light microscope, as supercoiling did not occur yet (18). Further, prophase can be discerned when sister chromatids become visible as a result of supercoiling, and when there is a nuclear envelope encapsulating the visible sister chromatids. In metaphase, the nuclear envelope breaks, and sister chromatids align along the equator where the spindle microtubules attach to the centromeres (18). In anaphase, the centromere breaks, and the sister chromatids are pulled to opposite poles of the cell. Telophase can be recognized by the formation of a nuclear membrane surrounding the newly separated chromosomes, as well as loss of visible chromosomes as a consequence of uncoiling (18, 19). Finally, during cytokinesis, a cell wall forms along the equator, separating the cell into two individual cells. Cells in mitosis (prophase, metaphase, anaphase and telophase) were considered mitotic cells and cells in interphase (G_1, S & G_2) and cytokinesis were deemed non-mitotic cells (19).

Statistics

The Statistical Package for the Social Sciences (SPSS) program produced p-values that were statistically significant at 95% confidence for the 4-hour and 5-hour populations. The remainder of populations were characterized as non-significant based upon the p-values.

To ensure the data met the assumptions of the paired samples t-test, several statistical tests were carried out beforehand. Normality was tested through the application of Normal Q-Q plots, Kolmogorov-Smirnov2 normality tests, Shapiro-Wilk normality test, skewness values and kurtosis values. To test for outliers, a box and whisker plot was utilized. All statistical descriptors were generated using SPSS.

Received: August 5, 2019
Accepted: May 7, 2020
Published: June 9, 2020

REFERENCES

1. Benca, Jeffrey P., Ivo AP Duijnstee, and Cindy V. Looy. "UV-B–induced forest sterility: Implications of ozone shield failure in Earth's largest extinction." *Science Advances*, vol. 4, no. 2, 2018, pp. 16-3. DOI. 10.1126/sciadv.1700618
2. Caldwell, Martyn M. "Solar UV irradiation and the growth and development of higher plants." *Photophysiology*, vol. 6, no. 1, 1971, pp. 131-177. DOI. 10.1007/BF00349807.
3. Caldwell, M. M., *et al*. "Action spectra and their key role in assessing biological consequences of solar UV-B radiation change." *Stratospheric ozone reduction, solar ultraviolet radiation and plant life,* vol. 6, no. 1, 1986, pp. 87-111. DOI. 10.1039/c0pp90035d
4. Flint, Stephan D., and Martyn M. Caldwell. "A biological spectral weighting function for ozone depletion research with higher plants." *Physiologia plantarum*, vol. 117, no. 1, 2003, pp. 137-144. DOI. 10.1032/BF0383922
5. Reboredo, Fernando, and Fernando JC Lidon. "UV-B radiation effects on terrestrial plants-A perspective." *Emirates Journal of Food and Agriculture*, vol. 1, no. 2, 2012, pp. 502-509. DOI. **doi**.org/10.9755/ejfa.v24i6.14670
6. Jingjing, Xu, *et al*. "Effects of Enhanced UV-Radiation on Morphological and Physiological Characters of Welsh Onion [J]." *Chinese Agricultural Science Bulletin*, vol. 11, no. 2, 2008, pp. 120-134. DOI. 10.1016/**j**.plaphy.2016.04.004
7. Shimotohno, Akie, *et al*. "The plant-specific kinase CDKF; 1 is involved in activating phosphorylation of cyclin-dependent kinase-activating kinases in Arabidopsis." *The Plant Cell*, vol. 16, no. 11, 2004, pp. 2954-2966. DOI. 10.1105/tpc.104.025601
8. Coohill, Thomas P. "Ultraviolet action spectra (280 to 380 nm) and solar effectiveness spectra for higher plants." *Photochemistry and photobiology*, vol. 50, no. 4, 1989, pp. 451-457. DOI. 10.1016/1011-1344(92)80043-u
9. Lidon, Fernando JC, *et al*. "Impact of UV-B radiation on photosynthesis-an overview." *Emirates Journal of Food and Agriculture*, vol. 10, no. 4, 2012, pp. 546-556. DOI. 10.9755/ejfa.v24i6.14673
10. Hsu, Henry, and Peter A. Lachenbruch. "Paired t test." *Wiley encyclopedia of clinical trials*, vol. 1, no. 3, 2007, pp. 345-378. DOI. 10.1155/2014/604581.
11. Vanhaelewyn, Lucas, *et al*. "Hormone-controlled UV-B responses in plants." *Journal of experimental botany*, vol. 67, no. 15, 2016, pp. 4469-4482. DOI. 10.1155/2014/604581.
12. Davies, Peter J. "The plant hormones: their nature, occurrence, and functions." *Plant hormones*, vol. 1, no. 15, 2010, pp. 12-56. DOI. 10.1007/978-1-4020-2686-7_1.
13. Nawkar, Ganesh M., *et al*. "UV-induced cell death in plants." *International journal of molecular sciences*, vol. 14, no. 1, 2013, pp. 1608-1628. DOI. 10.3390/ijms14011608
14. Escobar-Bravo, Rocio, Peter GL Klinkhamer, and Kirsten A. Leiss. "Interactive effects of UV-B light with abiotic factors on plant growth and chemistry, and their consequences for defense against arthropod herbivores." *Frontiers in Plant Science,* vol. 27, no. 8, 2017, pp. 14-45. DOI. 10.1080/15592324.2019.1581560.
15. Kakani, V. G., *et al*. "Field crop responses to ultraviolet-B radiation: a review." *Agricultural and forest meteorology,* vol. 120, no. 4, 2003, pp. 191-218. DOI. 10.1016/j.agrformet.2003.08.015
16. Kataria, Sunita, Anjana Jajoo, and Kadur N. Guruprasad. "Impact of increasing Ultraviolet-B (UV-B) radiation on photosynthetic processes." *Journal of Photochemistry and Photobiology B: Biology,* vol. 137, no. 1, 2014, pp. 55-66.
17. Xiong, Fusheng S., and Thomas A. Day. "Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants." *Plant Physiology,* vol. 125, no. 2, 2001, pp. 738-751. DOI. 10.1104/pp.125.2.738
18. Rozema, J., *et al*. "The role of UV-B radiation in aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-absorbing compounds." *Journal of Photochemistry and Photobiology B: Biology,* vol. 66, no. 1, 2002, pp. 2-12. DOI. 10.1029/2002PA000853.
19. González-Fernández, A., *et al*. "A model for dynamics of cell division cycle in onion roots." *Protoplasma,* vol. 65, no. 3, 1968, pp. 263-276. DOI. 10.1104/pp.116.4.151