The effect of UV irradiation on *Vigna radiata* seeds' (mung beans) germination and growth

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Abstract

This experiment examined the effect of UVA/UVB irradiation of *Vigna radiata* germination and on the growth of the radicle/hypocotyl of the seeds, thereby testing the effects of global warming and climate change on modern agriculture. The effect of UV radiation on the depletion of the ozone layer has been thoroughly examined for many decades, thus catalyzing global warming [9]. There were three treatments: control, low UVA/UVB and high UVA/UVB exposure. Germination percentage and length of the radicle data was measured over a period of 48 hours to see whether the UVA/UVB irradiation promotes or inhibits germination time and growth. In this experiment, germination was defined as the first evidence of the emergence of a radicle and the length of the radicle was measured at 12-hour time intervals. It was hypothesized that UV radiation will speed up germination but prolonged exposure will inhibit or hinder the growth of the seeds. In support of the hypothesis, the results showed that low UVB radiation does have a positive effect on the average length of a radicle. However, at high UVB, it was found that growth was inhibited.

***K*eywords** Ecology · *Vigna radiata* · Germination · UV Irradiation · Climate Change · Agriculture

1 Introduction

Climate change is currently at the forefront of recent political debate and is one of the most pressing concerns many people have today. Many individuals have taken a stronger stance on this subject, as this is one of the most problematic issues facing our generation. The Annual Review of Environment and Resources has found that food systems, which include the production, processing and transportation of food, contribute about 19-29% of the greenhouse gas emissions (GHG) in the atmosphere [9]. Likewise, agricultural activities (like using fertilizers) also contribute to the degradation of our ozone layer [9].

There has been a strong push to ban certain fertilizers but this effort has not been effective since many countries around the globe still use (chlorofluorocarbons) CFC and nitrogen fertilizers for their crop production. The usage of these fertilizers and the extent to which they contribute to the depletion of the ozone layer has been well documented in recent research [9]. This in turn will allow an increased amount of harmful radiation (radiation with shorter wavelengths) to penetrate the atmosphere. One of the biggest challenges farmers face is the impacts of climate change on crop yields. The negative effect of an increase in radiation exposure on agriculture may threaten the world's food supply [6].

Background

Ozone is the main component of an atmospheric layer that shields the Earth from harmful UV radiation exposure. The ozone layer blocks all UV-C radiation and some of the UV-B radiation [1]. However, the increased usage of CFCs in several countries around the world has shown to enhance the depletion of the ozone layer, which affects Canadian farmers and exposes their crops to more UVB and UVC radiation [6]. CFCs are organic molecules that decompose to their components through combustion, and the resulting increase in chlorine radicals is what breaks down the ozone layer with the exposure of UV light. The wavelengths then have the ability to penetrate the ozone layer and expose the world to harmful radiation through the atmosphere.

There have been many studies that investigated the affect of UV radiation on plant cells. An example is a study conducted by Hollósy involving a comprehensive analysis of the affects of UV radiation on different parts of the plants (including seedlings) and the processes involved in the growth of the plant[5]. Hollósy tested and recorded the effects on plant cells' nucleic acids, proteins, amino acids, proteins, pigment production, membranes, photosynthesis, structural modifications and plant-growth regulators [5].

Hollósy found that UV radiation results in the destruction of some amino acid residues of proteins, leading to inactivation of many enzymes [5]. An enzyme that could be affected by UV radiation is amylase, which is responsible for breaking down starch and is used as the carbon source for germination [8]. If amylase is inactivated, germination will not occur.

Under natural conditions, there are many inhibitors to germination that must be leached by water in order for germination to proceed [8]. However, if these inhibitors are disrupted then germination will proceed at a faster rate [10]. Another possibility is that cell to cell cohesion would be weakened therefore allowing the radicle to rupture more easily [5]. Hollósy also found that the UV absorption within the protein matrix of the seed coat and causes damage to the absorption site in the micropyle via energy migration [5]. The absorption site is an important part in the process of germination as water needs to be absorbed to initiate the breakdown of starches that fuel germination and the growth of seed. If the absorption site is disrupted then water cannot be absorbed and the process of germination cannot occur.

Hollósy concluded in terms of growth that elongation was increased temporarily and attributed this trend to the increased production of a phytohormone, a chemical that promotes radial elongation in plant cells [5]. Another study conducted by Kovács and Keresztes' found that the mitochondria in plant cells had a temporary increase in activity of biological processes followed by a steep increase of "resistance to irradiation" in response to radiation[3].

Aim

The aim of this study is to investigate the effect of UVA/UVB exposure on the germination and growth of seedlings. We also want to determine whether or not UV exposure will induce germination at a quicker rate and how the light with the wavelengths in the UV spectrum affects the growth of the radicle.

Predictions

In terms of predictions of the outcome of the experiment, we have two expected outcomes for germination and growth of the radicle based on our research into the topic and preliminary trials.

1. **Germination -** In terms of germination, UV radiation would speed up germination because UV light is of lower wavelength than visible light and therefore has more energy. Consequentially, due to the weakening of cell to cell cohesion in the seed coat, the seed will rupture faster, therefore germination will proceed at a faster rate. While it could inactivate or impede the function of important enzymes, UV itself will be responsible for weakening of the seed coat allowing first evidence of germination to be seen.

2. **Growth -** UV radiation would inhibit or hinder the growth of radicle based on research by Kovács and Keresztes. The temporary increase in elongation would not be seen in this experiment due to the difference in time that the experiments were conducted for. Therefore, there will be overall resistance in terms of growth for UV irradiated seedlings.

2 Methodology

When selecting an appropriate seed for this experiment several tests were performed on different species of seeds to determine the best seed to get optimal results from this experiment. Various species of tomato and onion seeds were first tested, but the seeds from the supplier seem to be defective after 6 days as only a few germinated. It was decided to go with *Vigna radiata*, a seed that sprouts faster than 6 days but not too fast so it was possible for us to document the effect of the changes. After several pre-trials with *V. radiata*, commonly known as mung beans, these seeds were chosen for its fast germination and fast growth.

Figure 1: Setup of the control (left) and low UVA/UVB (right) treatments. The high UVA/UVB treatment in the other side of the room.

There were three treatments: seeds that were exposed to high UVA/UVB, low UVA/UVB and the control group (no special UVA/UVB treatment). The experimental setup is depicted in Figure 1 with the last treatment group in a different part of the same room. There were lamps with different bulbs emitting different intensities of UVA/UVB. The 26-watt light bulbs were placed 20cm above the Petri-dishes.

The control group had no lamp and therefore no exposure to UVA/UVB. All light was blocked out. The low UVA/UVB light bulb produced 37 $\mu W cm^{-2}$ of UVB and 110 $\mu W cm^{-2}$ of UVA, and the high UVA/UVB light bulb produced 64 $\mu W cm^{-2}$ of UVB and 185 $\mu W cm^{-2}$ of UVA. While these intensities of UV are considered high today, in the future they may become the norm due to the depletion of ozone therefore, to achieve the objective the highest UV intensity light-bulb available was used to showcase the effects of raised levels of UV on the seeds.

Each Petri dish had two circular pieces of paper towels that were dipped in water. On those paper towels five seeds were placed as shown in Figure 2. Fifteen such Petri dishes were used for each treatment. A pipette was used to transfer 15mL of water to each petri dish daily to keep the paper towels damp.

At periodic intervals of 12 hours, 24 hours 36 hours and 48 hours, the number of seeds germinated was recorded, and the length of the radicle of the germinated seeds was recorded. The length was measured using a string and a caliper, with an error $\pm 0.01mm$ due to curvature of the radicle (Fig. 2), making it more difficult to take accurate measurements using just a ruler. The temperature of the room was measured daily and fluctuated from $25 - 27°C$ between morning and evening—however, all experiments were exposed to the same conditions so there should be no effect of temperature on the interpretation of results.

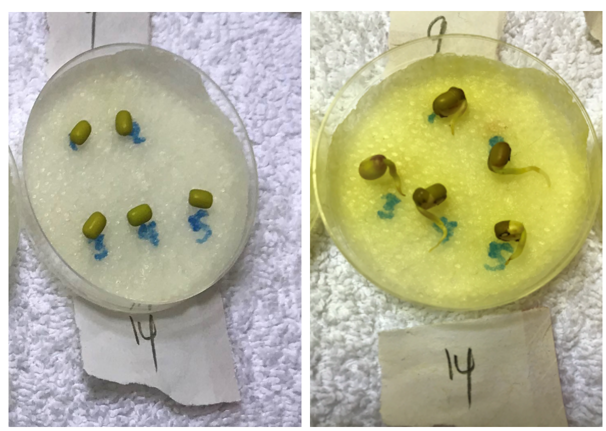

Figure 2: A picture of the *Vigna radiata* seeds before (left) and after (right) 48 hours of high UVA/UVB treatment.

The normality of the successful germination and radicle growth was tested using the Shapiro-Wilk normality test and because the data was not normally distributed, non-parametric Kruskal-Wallis tests paired with Dunn's post hoc was then run to determine which groups in each time stamp were significantly different.

All figures were created and all statistical tests were conducted in R (version 3.6.2).

3 Results

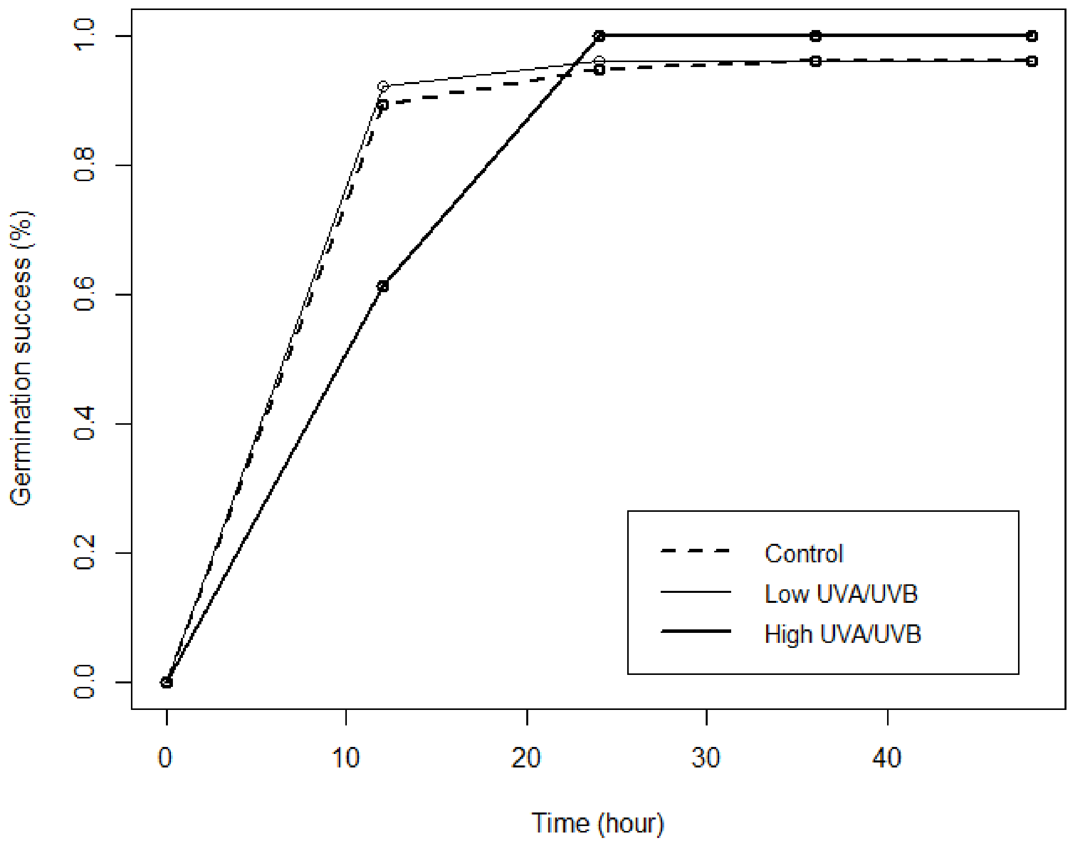

Figure 3: Germination success percent of *Vigna radiata* over 48 hours in standard UVA/UVB ($n = 72$), low UVA/UVB ($n = 72$) and high UVA/UVB ($n = 75$). $p = 0.124229$.

Table 1: Germination success percent of *Vigna radiata* at 12, 24, 36, and 48 hours in standard UVA/UVB ($n = 72$), low UVA/UVB ($n = 72$) and high UVA/UVB ($n = 75$). $p = 0.124229$.

Time (hour)	Control	Low	High
12	0.893	0.920	0.613
24	0.947	0.960	1.000
36	0.960	0.960	1.000
48	0.960	0.960	1.000

As shown in Table 1, in the control samples, 89% of the seeds germinated over 12 hours, 95% in 24 hours, and 96% after 36 hours. No more seeds germinated after 36 hours. In the low UVA/UVB samples, 92% of the seeds germinated over 12 hours, and 96% of the seeds germinated over 24 hours. No more seeds germinated after 24 hours. In the high UVA/UVB sample, only 61% of the seeds germinated over 12 hours, but after 24 hours all the seeds were germinated. There was not a significant effect of UVA/UVB irradiation on seed germination percentage as determined by the Kruskal-Wallis test (Chi-squared $= 2.1832, df = 2, p = 0.3357$).

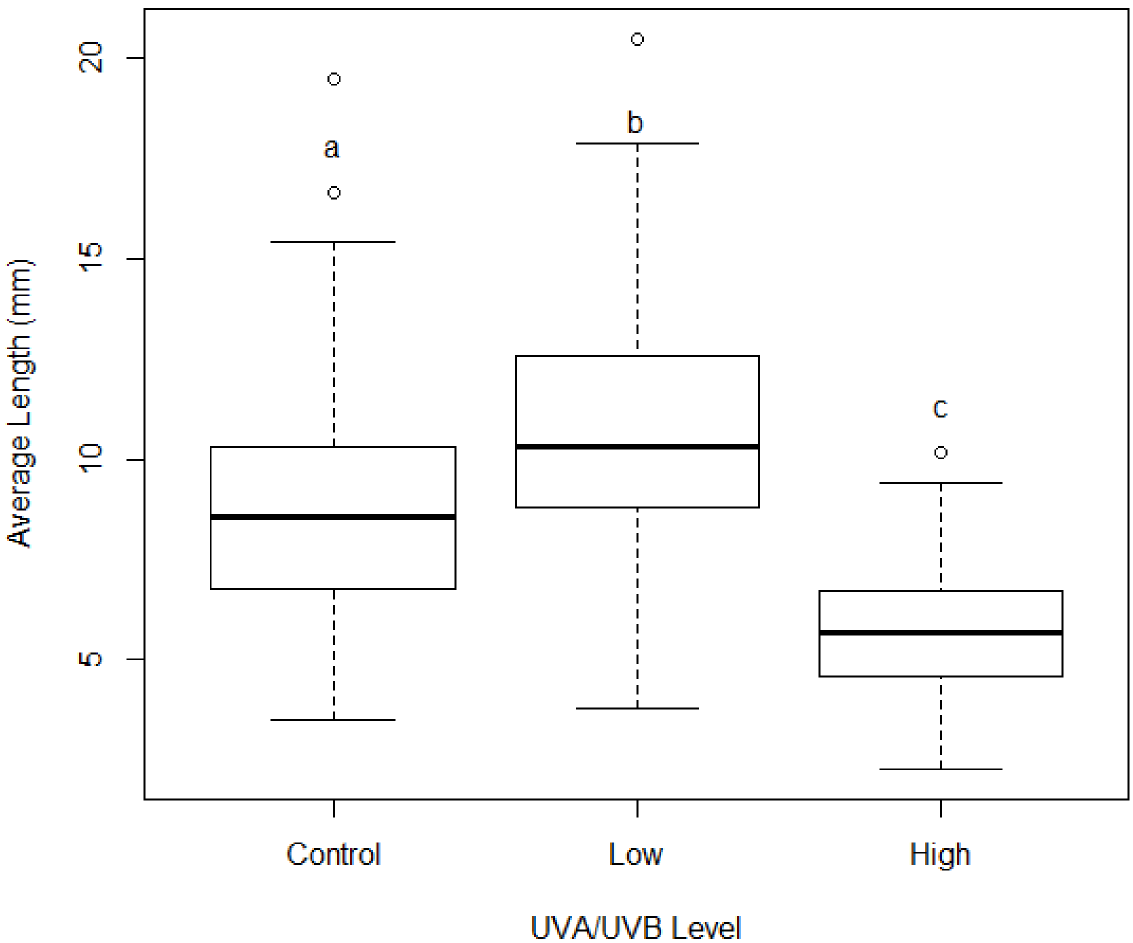

Figure 4: Average length (mm) of *Vigna radiata* recorded at 48 hours in control UVA/UVB ($n = 74$), low UVA/UVB ($n = 73$), and high UVA/UVB ($n = 75$). Box = 25th and 75th percentiles; bars = min and max values. $p < 2.2 \times 10^{-16}$.

The mean (±95% CI) length of the radicle of *V. radiata* after 48 hours was 8.93±0.78 mm at control UVA/UVB (n = 74), 10.66±0.76 mm at low UVA/UVB (n = 73), and 5.76±0.36 mm at high UVA/UVB (n = 75). There was a significant effect of UVA/UVB irradiation on seed radicle growth as determined by the Kruskal-Wallis test (Chi-squared $= 89.468, df = 2, p < 2.2 \times 10^{-16}$; Figure 4). Post hoc comparisons using Dunn's post hoc indicated that all three groups differed significantly at $p < 0.01$.

The figures for 12, 24, and 36 hours of growth are included in the appendix, as **Figure 6**, **Figure 7**, and **Figure 8** respectively.

Taken together, these results suggest that UVA/UVB irradiation aids the growth of *V. radiata* seeds. Specifically, these results suggest that low UVA/UVB irradiation helps the seeds grow at a faster pace, but does not necessarily help it germinate faster. However, it should be noted that high UVA/UVB levels hinder seed germination rate and seed radicle growth.

4 Discussion

The objective of this study was to determine the effect of UVA/UVB irradiation on *V. radiata*, commonly known as mung beans, as measured by the germination success and growth of the radicle.

The results showed that for germination success, low UV exposure promoted the germination of the seeds at a faster rate compared to the control. However, high UV exposure delayed the germination. These results were similar to the second measurement, the growth of the radicle. The low UV exposure promoted the growth of the radicle. At periodic intervals it was found that the low UV treatment seeds had longer radicles compared to control and the high UVB seeds had shorter radicles overall.

It was predicted that the *V. radiata* would germinate faster and grow slower under UV exposure because UVA and UVB photons (280-315 nm) are of a lower wavelength than visible light therefore it has a more damaging effect on the seed coat. UV exposure disrupts the cell to cell cohesion to allow the absorption of water and moisture and this also allows the seed to rupture more easily [4]. Once water is absorbed, this initiates the breakdown of important starches by growth-promoting hormones in the endosperm and this is what fuels germination and the formation of the radicle. This allows the germination to proceed and at a quicker rate. UV exposure also indirectly affects growth by inactivating proteins involved in seed growth and intricate system of hormones. This in turn may impede the functionality of the proteins therefore delay growth of the radicle.

The degradation on the cell wall structure and functionality of plant cells with gamma irradiation has been thoroughly studied. A study by Kovács and Keresztes concluded that increasing radiation has shown the increased dissolution of the middle lamella of the cell wall [3]. It was found that that high UV exposure delayed the process of germination which does not supports the prediction, as the cell wall should have been weakened making it easier for the seed to germinate. The results is in opposition to the prediction which suggests that there may be other factors (proteins, enzymes, hormones) involved in germination affected by UV radiation. This may cause a delay in germination.

The low UVB growth (length of radicle) data does not support the prediction. It was found that the radiation promoted the growth of mung beans. This can be explained in Kovacs and Keresztes' study where they found that the mitochondria response to gamma radiation was a temporary increase in activity of biological processes followed by a steep increase of "resistance to irradiation" [3]. This could explain the increase in growth length of the radicle. This hypothesis can be further explored by having more periodic intervals and extending the time window from 48 hours to a couple days to observe the changes that occur.

The high UVB growth supported the prediction and led us to believe that there must exist a barrier in which the amount of radiation exposure inhibits growth of the radicle. That barrier was crossed and the increased UV exposure stimulated the increase in biological activity faster. Therefore the increase in biological activity was not seen due to limited time intervals of collected data. This is a potential limitation of the results.

These results are consistent with that of Noble where he tested cabbage, radish and agave seeds under exposure to UVB emitting light bulbs[4]. He conducted a similar experiment by having the seeds in the Petri dishes with water and having treatments exposed to ultraviolet light[4]. He found more rapid germination of seeds - however, prolonged exposure to UV was damaging and inhibited vegetative growth[4]. Another study by Peykarestan, Seify, Fadaei, and Hatim conducted a more realistic experiment in which they sowed the seeds in pots with soil, rather than Petri dishes, with treatments of UV rays (5 doses with wavelengths ranging from 220 to 400nm)[2]. Each day the seeds were dug up and the number of seeds that germinated was recorded[2]. Again, it was found that longer wavelengths (low UV intensity) promoted germination at the beginning. As time went on, the growth was inhibited[2].

The major source of limitation in this experiment is that the conditions of the experiment did not mimic the conditions of the natural terrain in Canada, and in turn that may have affected germination and growth of *V. radiata*. There are many abiotic and biotic factors that could affect the germination and growth of these seeds, which includes the amount of rainfall, presence of pesticides, predation by animals, to name a few. A study performed by Koger, Reddy and Poston analyzed the effect of temperature, light intensity, prechilling, pH, osmotic and salt stress, planting depth and amount of water on *Caperonia palustris* seeds, also known as texasweed [7]. The results of this study did indeed show that these factors have an effect on the germination and growth of these seeds[7]. Therefore, it is not plausible to extrapolate these findings to the natural environment in Canada and make the correlation between climate change and crop yields.

In terms of improvements, there are certainly areas where it is possible to improve the data set. By extending the data collection by a couple of extra days and have more periodic intervals, it would provide a confirmation of Kovacs and Keresztes' results. Doing so could determine, with more specificity, the time during the process of germination that had the most impact from the UV radiation. Also, in future investigations, amending the methodology to mimic a more realistic situation as described in [2] by having pots instead of Petri-dishes will help us examine the more practical implications of UV radiation on farming and agriculture.

5 Conclusion

In conclusion, our experimental findings did indeed comport with the scientific literature on this topic and achieved its purpose in determining and verifying the effect of UV exposure on growth and germination of *V. radiata* seeds. The findings were that the high UV treatments delayed germination in comparison to the control and low UV treatment. After germination, the low UV exposure increased growth of the radicle over the 48 hours whereas high UV exposure decreased the growth of radicle. Germination success data was not statistically significant, which shows that there was no significant difference—however the length of the radicle data was statistically significant. Our study confirmed that high UVB levels can inhibit early-stage growth of *V. radiata* seeds. Future research and amendments to the methodology will help determine whether growth-inhibition persists beyond 48 hours or occurs in the natural environment. The findings of this study have an important implication in our society - we must do something to tackle climate change or we may face a shortage in the world's food supply in our future.

6 Acknowledgments

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7 Appendix

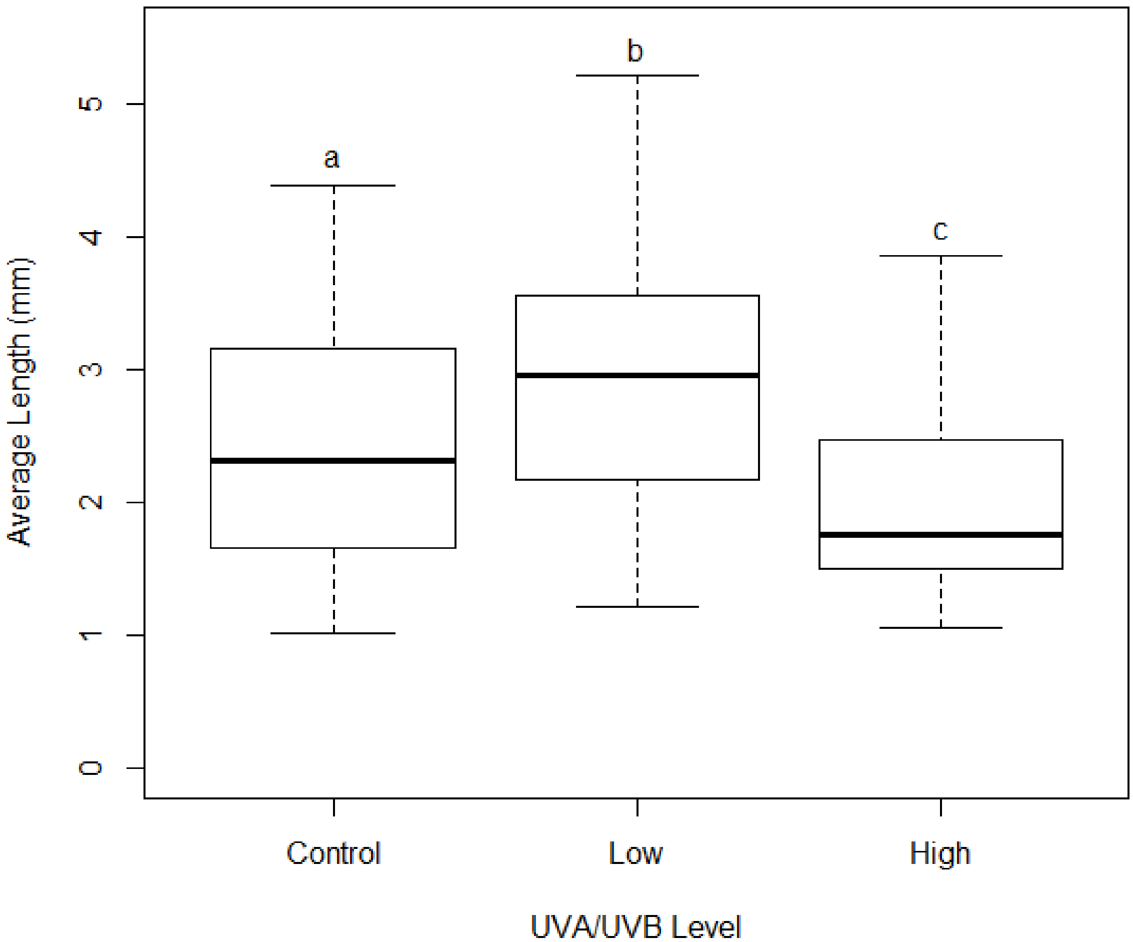

Figure 5: Average length (mm) of *Vigna radiata* recorded at 12 hours in control UVA/UVB ($n = 67$), low UVA/UVB ($n = 69$), and high UVA/UVB ($n = 46$). Box = 25th and 75th percentiles; bars = min and max values. $p = 7.697 \times 10^{-7}$.

At 12 hours of exposure, the mean (±95% Confidence Interval (CI)) length of the radicle of *V. radiata* at low UVA/UVB (2.91±0.23 mm) was greater than at control UVA/UVB (2.40±0.21 mm), but the length at high UVA/UVB (1.97±0.18 mm) was shorter than the control. There was a significant effect of UVA/UVB irradiation on seed radicle growth as determined by the Kruskal-Wallis test (Chi-squared $= 28.154, df = 2, p = 7.697 \times 10^{-7}$; Figure 6). Post hoc comparisons using Dunn's post hoc test indicated that all three groups differed significantly at $p < 0.01$.

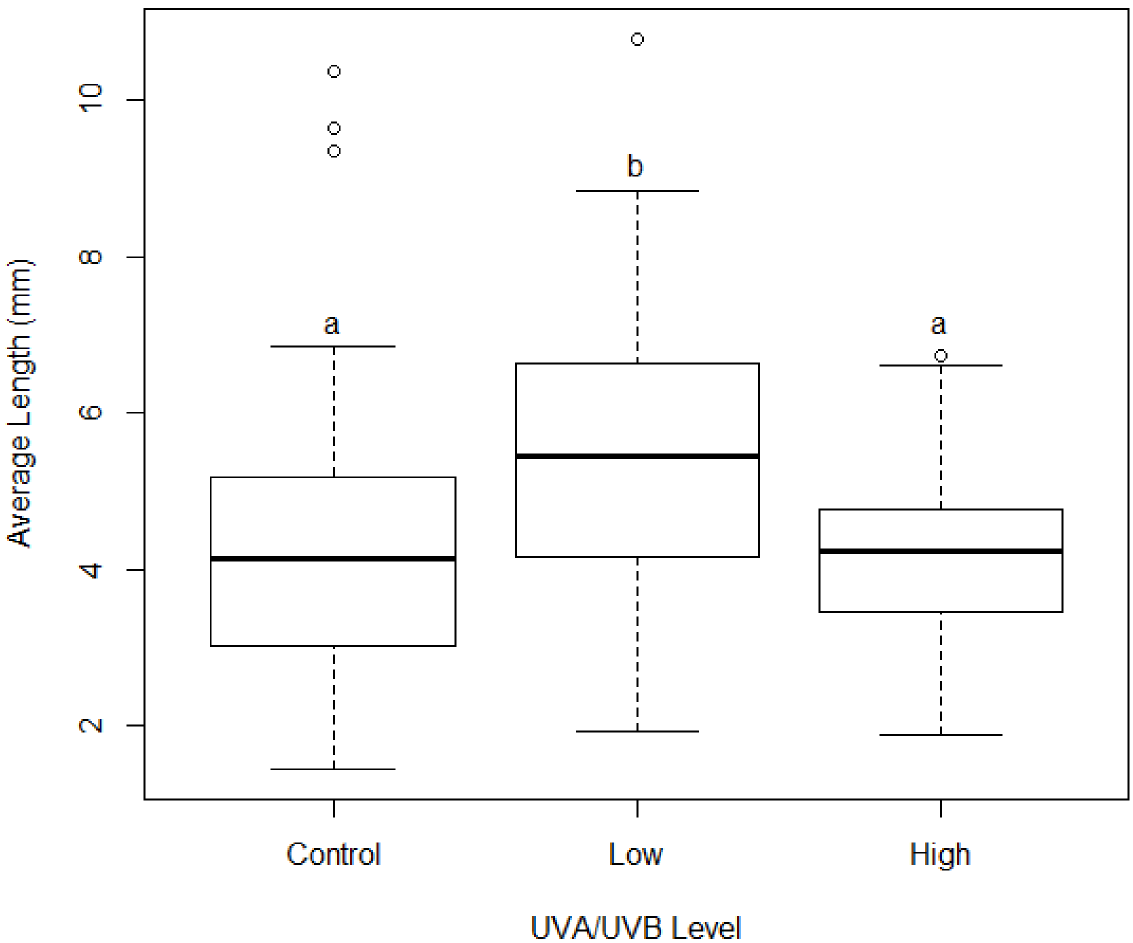

Figure 6: Average length (mm) of *Vigna radiata* recorded at 24 hours in control UVA/UVB ($n = 73$), low UVA/UVB ($n = 73$), and high UVA/UVB ($n = 75$). Box = 25th and 75th percentiles; bars = min and max values. $p = 2.787 \times 10^{-6}$.

At 24 hours of exposure, the mean (±95% CI) length of the radicle of *V. radiata* at low UVA/UVB (5.42±0.40 mm) was greater than at control UVA/UVB (4.30±0.42 mm), but the length at high UVA/UVB (4.20±0.23 mm) was about the same as the control. There was a significant effect of UVA/UVB irradiation on seed radicle growth as determined by the Kruskal-Wallis test (Chi-squared = 25.581, $df = 2, p = 2.787 \times 10^{-6}$; Figure 7). Post hoc comparisons using Dunn's post hoc test indicated that the control vs. high UVA/UVB and low UVA/UVB vs. high UVA/UVB groups differed significantly at $p < 0.01$. However, the control did not significantly differ from the high UVA/UVB ($p = 0.80$).

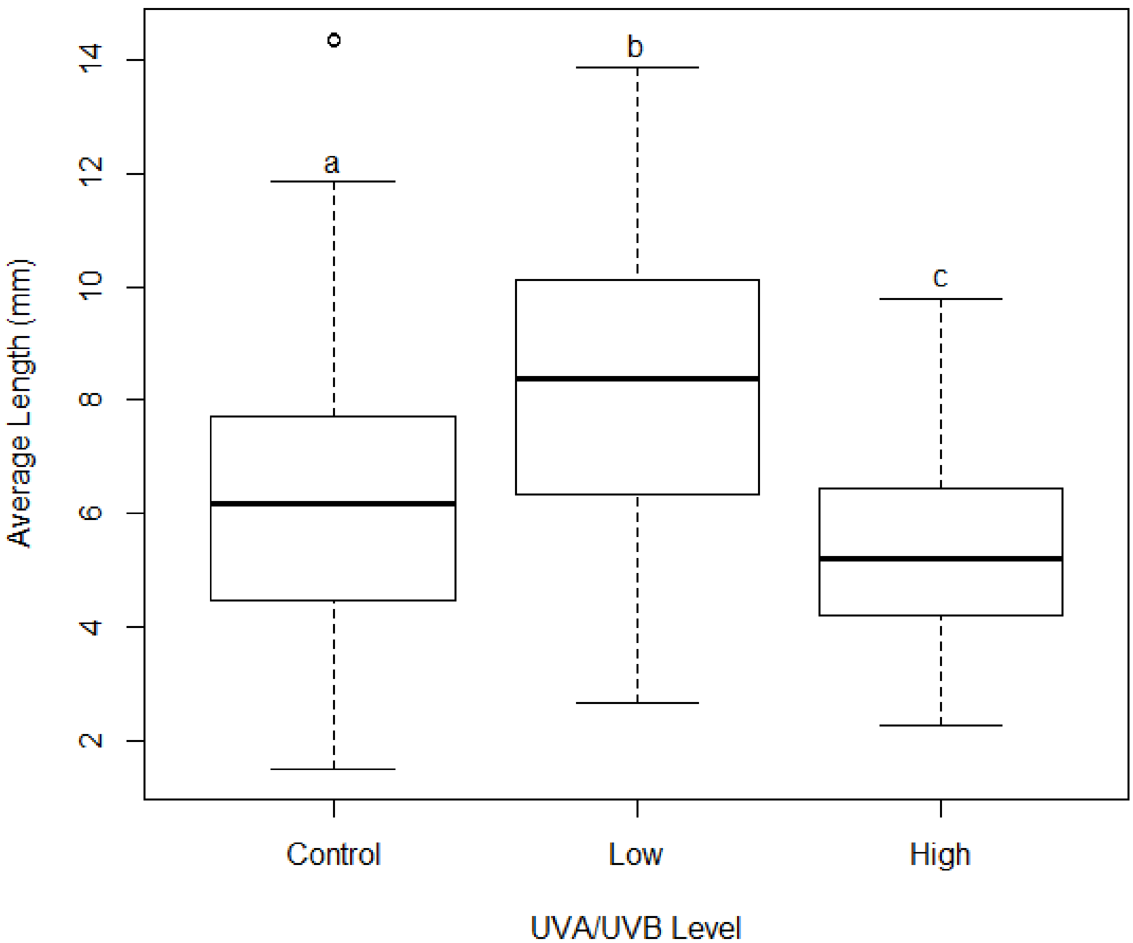

Figure 7: Average length (mm) of *Vigna radiata* recorded at 36 hours in control UVA/UVB ($n = 74$), low UVA/UVB ($n = 73$), and high UVA/UVB ($n = 75$). Box = 25th and 75th percentiles; bars = min and max values. $p = 9.244 \times 10^{-11}$.

At 36 hours of exposure, the mean (±95% CI) length of the radicle *V. radiata* at low UVA/UVB (8.32±0.64 mm) was greater than at control UVA/UVB (6.44±0.64 mm), but the length at high UVA/UVB (5.37±0.36 mm) was shorter than the control. There was a significant effect of UVA/UVB irradiation on seed radicle growth as determined by the Kruskal-Wallis test (Chi-squared $= 46.209, df = 2, p = 9.244 \times 10^{-11}$; Figure 8). Post hoc comparisons using Dunn's post hoc test indicated that all three groups differed significantly at $p < 0.01$.