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UV-B and UV-C radiation on the germination of soybean seeds

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ABSTRACT: UV radiation use is currently receiving attention due to concerns about its potential positive or negative effects on the physiological responses of plants, so the goal of this study was to evaluate the influence of natural and artificial ultraviolet radiation (UV-B and UV-C) on soybean seed germination. Initially, the seeds were exposed to UV radiation for different periods of time (0, 1, 2, 4, and 8 hours), with a radiation intensity of 2.5 W m^{-2}. They were sown on germitest paper and stored in a Biochemical Oxygen Demand chamber set at 25 °C and a 12-hour photoperiod. Tests were conducted for germination (at 8 days), first count (at 5 days), length, and dry mass of seedlings. The results indicated that exposure to UV-B and UV-C (from 1 hour - 9000 J m^{-2}) light reduced the germination percentage, the seedling length, and the dry mass of the soybean seedlings. The decrease in root length was observed after 2 (UV-C) and 4 hours (UV-B) of light exposure. It was concluded that, under the conditions of this study, exposing soybean seeds to UV-B and UV-C radiation influenced the percentage of germination and initial seedling growth.

Key words: *Glycine max*; light; process of germination; ultraviolet

Radiação UV-B e UV-C na germinação de sementes de soja

RESUMO: Atualmente, a radiação ultravioleta tem recebido maior atenção devido à preocupação com os possíveis impactos positivos ou negativos nas respostas fisiológicas das plantas. Neste contexto, o objetivo deste estudo foi avaliar a influência da radiação ultravioleta (UV-B e UV-C) na germinação de sementes de soja. Inicialmente, as sementes foram submetidas a diferentes tempos de exposição à luz UV (0, 1, 2, 4 e 8 horas) com intensidade de radiação 2.5 W m^{-2}. Em seguida, foram semeadas em papel germitest e mantidas em câmara BOD, a 25 ºC e fotoperíodo de 12 horas. Foram realizados os testes de germinação (aos 8 dias), primeira contagem (aos 5 dias), comprimento e massa seca de plântulas. Os resultados indicaram que a exposição à luz UV-B e UV-C (a partir de 1 hora - 9000 J m^{-2}) reduziu a percentagem de germinação, o comprimento e a massa seca da parte aérea das plântulas de soja. O decréscimo do comprimento radicular foi observado a partir de 2 (UV-C) e 4 horas (UV-B) de exposição à luz. Conclui-se que, nas condições deste estudo, a exposição das sementes de soja à radiação UV-B e UV-C afetou a porcentagem de germinação e o crescimento inicial das plântulas.

Palavras-chave: *Glycine max*; luz; processo germinativo; ultravioleta

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Introduction

Soybean is the most important agricultural crop in Brazil, with an estimated production of 135.9 million tons of grain in the 2021/22 crop year (Conab, 2021). Consequently, soybeans play a crucial role in Brazil trade balance and are one of the world most important commodities. This oilseed is gaining prominence due to the constant global population growth and its multiple uses, including as a source of vegetable protein in human and animal food, as well as the production of biofuels (Jo et al., 2022).

One of the main steps determining the success of the implantation of areas with cultivated species is the germination of the seeds. Several factors influence the germination process, including exposure to light. Light has been used to control morphogenesis, photosynthesis, growth, and to promote resistance to photon flux damage in plants that receive light at different wavelengths, intensities, and exposure times (Anders & Essen, 2015). The light is identified by its wavelength within the electromagnetic spectrum, such as ultraviolet - UV (A: 315-400 nm, B: 280-315 nm and, C: 100-280 nm), with only UV-A and UV-B reaching the earth's surface. UV light can influence the physiological responses of plants since intensity, wavelength, and exposure have a significant impact on plant growth and development (Loconsole & Santamaria, 2021).

The effect of ultraviolet radiation on seed biology and germination is poorly understood (Rupiasih & Vidyasagar, 2016). It is known that seeds respond to UV radiation at wavelengths found in sunshine (UV-A and UV-B) and below 280 nm (UV-C). Several studies have been conducted that indicate either favorable or negative impact of UV-B and UV-C light on seed germination and seedling growth in a wide range of crops, such as wheat (Rupiasih & Vidyasagar, 2016; Semenov et al., 2020), soybean, wheat, pine and sunflower (Pournavab et al., 2019), maize and sugar beet (Sadeghianfar et al., 2019), barley (Lazim & Ramadhan, 2020), bean (Hernandez-Aguilar et al., 2021), *Arabidopsis thaliana* (Zhang et al., 2020), *Pinus nigra* (Ozel et al., 2021), *Vicia vilosa* (Semenov et al., 2021), and *Allium cepa* (Çavuşoğlu et al., 2022).

However, at the moment, information on the possible application of radiation for seed treatment is insufficient (Semenov et al., 2020). For this reason, identifying the possible effects of UV radiation on plants is crucial, as much as to understand the importance of the subject as to make the necessary decisions to determine the possible future effects (Ozel et al., 2021). Thus, this study aimed at evaluating the influence of ultraviolet radiation (UV-B and UV-C) on the germination of soybean seeds.

Materials and Methods

The research was conducted at the Universidade Federal de Santa Maria (UFSM), at the Laboratory of Plant Genetics, Department of Biology, Brazil, with soybean seeds [*Glycine max* (L.) Merrill], cultivar BMX Fibra IPRO, crop 2021.

Before every procedure, the lamps were on for five minutes. Then, the seeds were placed in Petri dishes and inserted into an irradiation chamber with a lamp emitting UV-B radiation (Ushio G15T8E) at a distance of 25.5 cm from the lamp, for different intervals of exposure to the light (0, 1, 2, 4, and 8 hours), according to Table 1. At a distance of 34 cm, the identical operation was carried out using a UV-C radiation lamp (Philips TUV 15WG15T8). The UV-B radiation intensity was calibrated using a UV Monitor MS-211-1 from EKO Instruments Co. Ltd., and the UV-C intensity was calibrated with a UV-C 254 radiometer from Lutron. It was essential to adapt the distance between the base and the UV-B and UV-C lamps to ensure the seeds got the same amount of radiation intensity (2.5 W m^{-2}).

The seeds were placed inside a glass Petri dish as spread out as possible to avoid overlapping each other and ensure that they were reached by the same intensity of radiation. The intensity of UV radiation was measured in different regions of the irradiation chamber using radiometers (models described above). The constant and highest intensity value was observed in the central region. The Petri dish with the seeds was placed in the central part of the chamber during the exposure times (treatments), ensuring that they were reached by the same intensity of UV light (Figure 1).

The physiological potential of the seeds was assessed by subjecting the seeds to different amounts of UV light.

Table 1. UV-B and UV-C doses and exposure times for soybean seed in a constant irradiance chamber.

Exposure time	Dose (J m^{-2})	Dose (mJ cm^{-2})
0 (control)	0	0
1 hour	9,000	900
2 hours	18,000	1,800
4 hours	36,000	3,600
8 hours	72,000	7,200

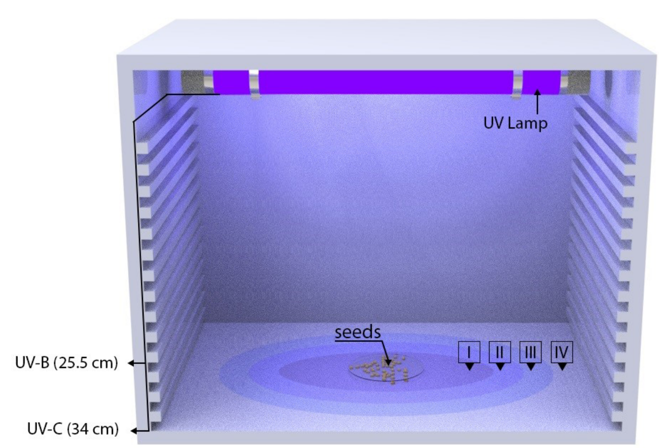

Figure 1. A representative scheme irradiation chamber shows seeds at the bottom and a UV lamp at the top. The indicators UV-B (25.5 cm) and UV-C (34 cm) on the left side show the level position where the seeds are left before installing the UV lamp (B or C). The intensity gradually decreases away from the center in the four areas (I, II, III, and IV) on the bottom center (I = 2.5 W m^{-2}, II = 2.1 W m^{-2}, III = 1.8 W m^{-2}, and IV = 0.7 W m^{-2}).

Soybean seeds were distributed on paper rolls and moistened with distilled water (at a rate of 2.5 times the paper weight). The paper rolls (four replications of 50 seeds for each time and condition of light) were stored in a Biochemical Oxygen Demand chamber after sowing, at a temperature of 25 °C, for 12 hours of light, and the counts were done on the 5th and 8th days, according to the adapted methodology of Brasil (2009). To assess the seedling length (cm), four replications of 20 seeds for each time (dose) and condition of light (UV-B and UV-C) were sown in two rows in the upper third of the germitest paper and maintained under the same condition as the germination test. On the 5th day after sowing, the lengths (shoots and root) of 10 normal seedlings of each replication were measured. Following the procedure, ten normal seedlings were chosen from each replicate of the seedling length test to determine the seedling and root dry mass (mg). The seedlings were weighed on a precision balance (of 0.001 g) after drying the material in a forced ventilation oven at 60 ± 5 °C for 48 hours (Krzyzanowski et al., 2020).

The experiment was carried out in a completely randomized design. The two treatments consisted of exposing the seeds to light (UV-B and UV-C separately) at five different times (doses) and four replications per treatment. The Shapiro-Wilk and Bartlett tests were used to confirm the homogeneity of variances and the normality of residuals, respectively. The data was submitted to an analysis of variance using the F ($p \leq 0.05$) test and, when significant, a regression analysis was performed using the program Sisvar software version 5.6. The mathematical models were selected based on the significance of the regression coefficients using the t test ($p \leq 0.05$), the coefficient of determination ($r^2 > 0.60$) and the significance of the mathematical model ($p \leq 0.05$).

Results and Discussion

Germination under UV-B radiation exposure

Through the analysis of the data, a significant difference was observed in the variables such as germination, first count, shoot and root length, and dry mass of soybean seedlings as a function of treatments (exposure times to UV light - B), according to Figure 2. The UV-B radiation exposure from 8 hours (72,000 J m^{-2}) was responsible for the reduction in the percentage of germination from 93% (control) to 80% and from 8 to 75% in the first count test (Figure 2A). In addition, there were no dead or hard seeds found in any of the treatments in which the seeds were exposed to radiation, only normal and abnormal seedlings.

After 8 hours of exposure to UV-B light, seedling length decreased from 5.5 cm (control) to 3.81 cm, while root length decreased considerably from 8.69 to 7.42 cm (Figure 2B). The decrease in root length was observed after 4 hours (36,000 J m^{-2}) of light exposure. The dry mass of the seedling were greater in the control treatment (no UV light exposure – 18.75 mg) than in all other treatments (15.61 mg – 72,000 J m^{-2}). However, there was no significant difference in root dry mass (Figure 2C).

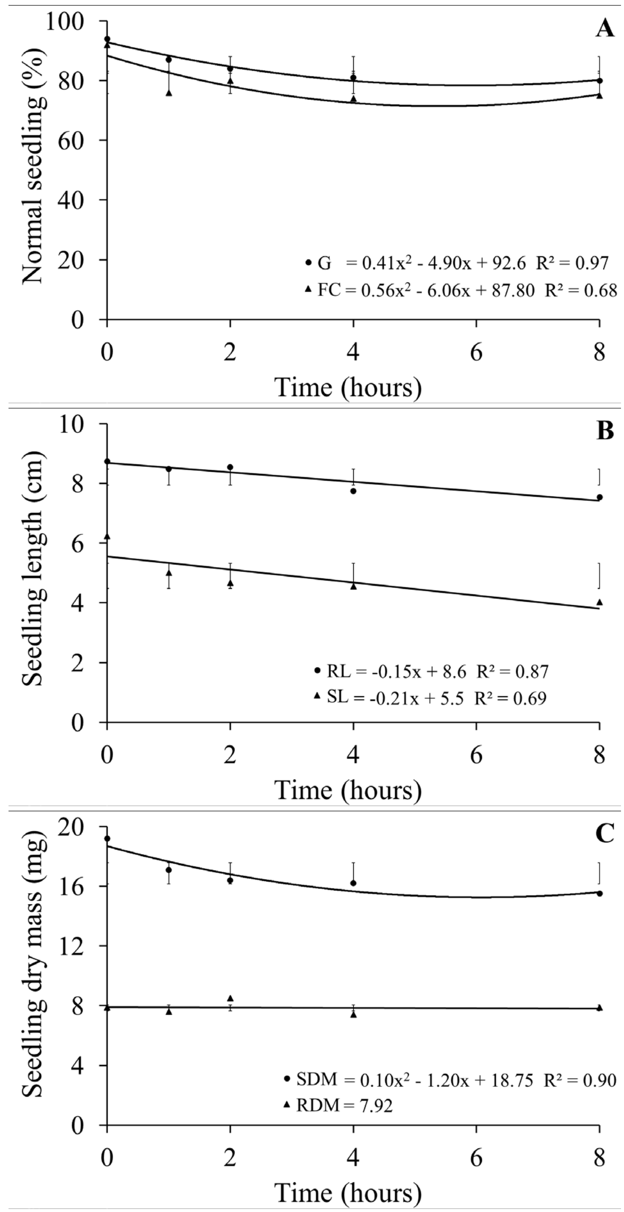

Figure 2. Germination and first count (A), shoot length (SL) and root length (RL) (B), seedling dry mass (SDM), and root dry mass (RDM) (C) of soybean seedlings exposed to UV-B radiation.

The results of this study corroborate those of Pournavab et al. (2019), who observed that soybean seeds had a low sensitivity to UV-B radiation (from 0 to 90 minutes and intensity 1.5 W m^{-2}) that increased as the radiation dose was increased, causing the mean seedling and root length to decrease proportionally to the amount of UV-B applied. The authors also found changes in the curvature of the hypocotyl and necrotic hypocotyl damage, which hindered the epicotyl from emerging. They also found cracks in the radicle.

In addition, Ma et al. (2018) analyzed four soybean varieties treated with different intensities of UV-B radiation (0, 5, 10, 20, and 40 μw cm^{-2}) and reported that the isoflavone concentration increased with UV-B radiation intensity and time but decreased with overtreatment. There was an inhibition of growth during germination, fresh weight, seedling length, free amino acids, and the content of reducing sugars with increasing exposure time to light. In addition, black spots on the cotyledons showed that there was damage to cells.

Complementarily, Ozel et al. (2021), analyzing *Pinus* seeds exposed to UV-B radiation throughout a range of times (from 5 to 60 minutes), found that germination and seedling characteristics were drastically affected and that, as the UV application time increased, these variables were negatively affected. In other studies, Zhang et al. (2020), suggested that UV-B radiation restricted meristem length and root elongation regions of *Arabidopsis thaliana* due to the reduced number of meristem cells. Furthermore, UV-B radiation at different exposure intervals (15, 30, and 45 minutes) resulted in a difference in the percentage of germination of *Scrophularia striata* seeds compared to the control treatment. Increasing UV exposure time reduced the germination of seeds significantly (Mousavi et al., 2022).

Furthermore, studies have shown that exposure to UV-B rays might have a harmful effect on the plant. These changes include genomic alterations, oxidative damage, lipid destruction, alterations in plant biochemistry, and slowed growth (Neugart & Schreiner, 2018). UV radiation is stressful for plants and has the impact of decreasing growth (Santos et al., 2021), consequently, even a slight increase in radiation can have significant biological effects.

Plants have a variety of mechanisms for dealing with UV-B stress that allows them to offset the negative effects of this radiation. One known prevention method used for plants to protect themselves against radiation exposure is the production of secondary metabolites (Azarafshan et al., 2020). Thus, the effects of UV-B light exposure should be investigated in issues such as the development of antioxidants, antifungals, antimicrobials, production of genotypes that will less suffer the effects of global climate change, and the development of individuals resistant to other stress factors (Ozel et al., 2021).

It is important to emphasize that UV-B radiation can also positively affect plants. It is known, for instance, that although radiation damages plant tissues, it can also promote the accumulation of UV-protective and antioxidant molecules in some plants used for food (He et al., 2019). Thus, it is possible to infer that, depending on the electromagnetic region employed, UV light treatment may have positive or negative effects (Romero-Galindo et al., 2021).

Finally, in the present investigation, it is possible to conclude that UV-B radiation at different exposure intervals resulted in a significant difference in soybean seed germination parameters compared to the control treatment (no UV light exposure). Probably, the ultraviolet light has interacted with the various structures of the seed, interfering with its metabolic processes and resulting in cell damage. This negative impact may have caused a reduction in root growth, consequently reflecting on the shoot length of soybean seedlings.

Germination under UV-C radiation exposure

Through the analysis of the data, a significant difference was observed in the variables such as germination, first count, seedling and root length, and seedlings dry mass of soybean as a function of treatments (exposure times to UV light - C), according to Figure 3.

Exposure to UV-C radiation from 8 hours (72,000 J m^{-2}) resulted in a linear reduction in the percentage of germination from 92% (control) to 77% and from 90 to 71% in the first count test (Figure 3A). In all treatments that received UV light, the seedling length and the seedling root length were shorter than the control (Figure 3B). Seedling length decreased from 6.10 cm (control) to 4.8 cm, while root length decreased considerably from 8.65 to 7.17 cm (Figure 3B). The decrease in root length was observed after 2 h (18,000 J m^{-2}) of light exposure. The dry mass of the seedling was greater in the control treatment, reducing from 18.91 to 15.54 mg (72,000 J m^{-2}). However, no significant difference in root dry mass was observed (Figure 3C).

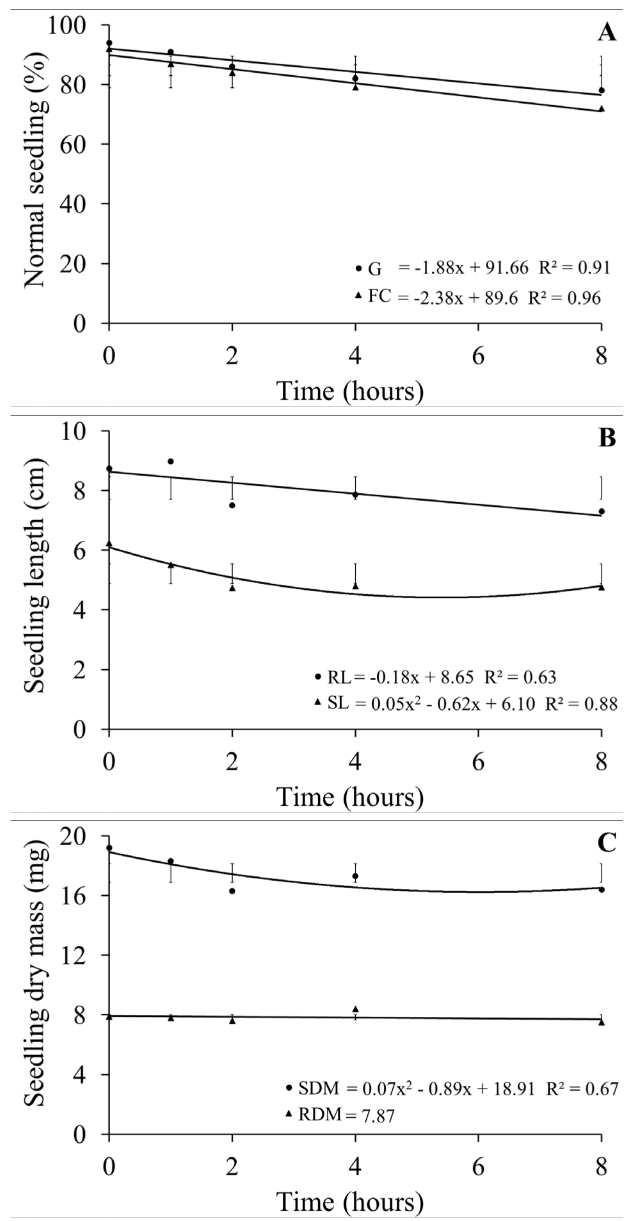

Figure 3. Germination and first count (A), shoot length (SL) and root length (RL) (B), seedling dry mass (SDM), and root dry mass (RDM) (C) of soybean seedlings exposed to UV-C radiation.

Rupiasih & Vidyasagar (2016) found similar results to this study when they exposed wheat seeds to UV-C light for 30 to 180 minutes. During all of these times, the shoot and root of the seedlings grew more slowly than they did in the control group. Additionally, Lazim & Nasur (2017) found that sorghum seedlings (*Sorghum bicolor* L.) exposed to UV-C for 30 and 60 minutes reduced germination speed and seedling length.

In a study with *Allium cepa*, Çavuşoğlu et al. (2022) reported that treatments with UV-C light (254 nm) caused a significant reduction in growth-related parameters. They suggested that exposure to UV light triggered growth inhibition, cytogenotoxicity, and damage to meristematic cells in the roots depending on the wavelength. In addition, anatomical damage was observed in the cells of the epidermis and cortex, necrotic zones, and nucleus with giant cells.

Furthermore, Hernandez-Aguilar et al. (2021), analyzing bean seeds exposed to UV-C light from 0 to 15 minutes (700 μw cm^{-2} intensity), found that radiation caused tissue deterioration, affecting seedling growth depending on the exposure time. UV light with shorter wavelengths has higher energy and can penetrate deeper into tissues (Çavuşoğlu et al., 2022). It is possible that ultraviolet light interacts with different structures of the seed and photostimulated chromophores intervene in its metabolic processes (Hernandez-Aguilar et al., 2021).

Complementarily, Pournavab et al. (2019) demonstrated that UV-C radiation (from 0 to 90 minutes and an intensity of 1.6 W m^{-2}) caused significant damage to germination and growth in *Pinus*, soybean, sunflower, and wheat. The authors concluded that soybean seeds showed low UV sensitivity at a dose of 43.2 kJ m^{-2}. Also, sunflower and *Pinus* exhibited a high sensitivity to UV doses due to the large concentration of lipids in their seeds, which would facilitate the deterioration of the plasma membrane.

Likewise, Araújo et al. (2020), using UV-C radiation doses corresponding to 0, 10.4, 20.7, 31.1, and 41.4 kJ m^{-2} (0, 15, 30, 45, and 60 minutes, respectively) and radiation intensity of 11.494 W m^{-2} verified that the dose of 41.4 kJ m^{-2}, despite showing the most significant reduction in the incidence of fungi, promoted reduction of vigor and viability of seeds, being harmful to their physiological quality.

Finally, according to the result of this research, different times of exposure to UV radiation (different doses) impacted the germination and initial development of soybean seeds. Although UV-B has significantly higher penetration power than UV-C, the latter is more genotoxic because its wavelength range is closer to the peak absorption of DNA and RNA (Hsu et al., 2021). However, the prolonged effects of UV radiation on plants are still not well understood, and more research is needed with different intensities or extended exposure periods to elucidate the physiological mechanisms involved and the effects on seed germination of other species that are important for food and the economy.

Conclusion

Under the conditions of this study, exposing soybean seeds to UV-B and UV-C radiation influenced the percentage of germination and initial seedling growth.

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Compliance with Ethical Standards

Author contributions: Conceptualization: RS, WJSG; Formal analysis: RS; Investigation: RS, RAMB, GLM, AHSR; Methodology: RS, RAMB, GLM, AHSR; Project administration: RS; Supervision: RS, WJSG; Validation: RS, RAMB, GLM, AHSR, WJSG; Visualization: RS, WJSG; Writing - original draft: RS, WJSG, LSD; Writing - review & editing: RS, RAMB, GLM, AHSR, WJSG, LSD.

Conflict of interest: The authors declare that there is no conflict of interest.

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