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Ultraviolet Radiation Effect on Seed Germination and Seedling Growth of Common Species from Northeastern Mexico

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Abstract: Light is the only key factor essential for plant growth, and ultraviolet (UV) rays, the harmful part of solar radiation, have the ability to decrease the metabolic rate in photosynthesis, causing deterioration in their growth. Accurate handling of the ultraviolet light that reaches the crops allows the improvement of different aspects of production, such as the shape and color of the plants, the precocity of the crops, and an improvement in the control of pests and diseases. The present study was carried out with the purpose of studying the responses in terms of germination and growth of four plant species—*Glycine max*, *Triticum aestivum*, *Helianthus annuus*, and *Pinus maximartinezii*—exposed to different doses of UV-C and UV-B radiation, for evaluation through the standard germination and accelerated aging tests, registering the germination rate (GR) and vigor (V), the number of normal seedlings (NS), and the average lengths of radicle (ALR) and plumule (ALP). Seeds treated with UV-C radiation showed a significant difference ($p < 0.05$) between treatments (doses) and species, both in the standard germination and accelerated aging tests for the ALR and ALP variables. Seeds of *G. max* showed low sensitivity in both tests, with the dose 43.20 kJ/m²/day. The seeds of *H. annuus* and *P. maximartinezii* showed high sensitivity in both tests under UV-C radiation, with the doses 8.64 kJ/m²/day and 0.864 kJ/m²/day, respectively. Both tests under UV-B radiation showed a significant difference ($p < 0.01$) between species and treatments in the variables NS and ALP, with the minimum dose (T2).

Keywords: Ultraviolet radiation; seed germination; seedling vigor

1. Introduction

Ultraviolet solar radiation has received greater attention during recent years, mainly due to concern for the depletion of stratospheric ozone [1] and the increase in UV-B radiation [2] that has detrimental effects on the growth and development of plants. Plants respond to light through different photoreceptors [3]. Apart from photosynthetically active radiation (PAR, 400–700 nm), plants are exposed to UV-A light (320–390 nm), UV-B (280–320 nm), and UV-C radiation below 280 nm [4].

Although it represents a greater portion of the total radiation, UV-A radiation is less harmful than the rest of wavelengths of UV radiation [5]. UV-B radiation of the ecologically relevant intensities is considered a modulator of physiological and morphological responses in plants [6]. It intervenes in

plant growth by affecting phytohormones through photodestruction or enzymatic reactions [7], and influences photoprotectors and photosynthetic processes [8]. As it is concerned, UV-C is the most energetic part of the UV spectrum, and is not found naturally in the biosphere, however, it is used artificially for its important bactericidal and germicidal action [9]. When it interacts with plant tissues, UV-C radiation causes damage by ionization and dimerization of pyrimidines in DNA molecules, as well as a decrease in protein synthesis and the alteration of their structures [10,11].

The induction of DNA damage by UV radiation is a key event that drastically influences the normal life processes of all organisms [9,12]. However, it is possible to obtain a beneficial effect from the sublethal application of an agent that induces physical or chemical stress [13] and at the same time induces the accumulation of compounds associated with stress, such as flavonoids and phenolics [14]. The accumulation of a series of phytoalexins has also been observed, as well as the typical adaptations of the induction of resistance, such as the modification of cell walls and even cell death [15]. Higher doses of UV-B or UV-C radiation result in cellular damage [5] and strong irradiation with UV-C exerts a series of negative effects [9]. The above refers mostly to photosynthetic tissues of seedlings and plants, as well as fruits and vegetables in post-harvest.

Fortunately, UV-C radiation is filtered by the ozone layer in the stratosphere, so the amount of this radiation reaching the Earth's surface is very low [16]. However, future UV-C radiation could increase as the result of stratospheric ozone depletion due to atmospheric pollution [17], highlighting ecological implications on natural ecosystems and agricultural productions [18]. In this way, it is crucial to study the effects of this radiation on some crops.

The information regarding irradiation of seeds with UV-C and UV-B is very scarce and refers in particular to the induction of tolerance to pathogens [19]. Therefore, this research seeks to determine the impact of UV-C and UV-B radiation in different doses, on the germination and vigor of seeds of *Helianthus annuus*, *Triticum aestivum*, *Pinus maximartinezii*, and *Glycine max*, in order to determine the minimum, maximum, and lethal doses of UV-B and UV-C irradiation and the application times for these species.

2. Materials and Methods

The species evaluated were soybeans (*Glycine max* L.) variety Huasteca from the locality INIFAP Tamaulipas 2010, wheat (*Triticum aestivum* L.) variety Gálvez from Navidad 2009, sunflowers (*Helianthus annuus* L.) variety commercial from Fraterna Primavera 2010, and pine (*Pinus maximartinezii* Rzedowski) from Cerro Piñones, Juchipila, Zacatecas.

2.1. UV-B and UV-C Radiation Doses

Preliminary tests were carried out to achieve the adequate doses (Table 1) at the right time, irradiating the seeds of each species with UV-C and UV-B for different exposure times (0, 5, 10, 15, 30, 60, and 90 min, until the limit of 29 h), according to the standard germination test [20,21]. The radiation intensity was kept constant at 1.6 W/m² and 1.5 W/m², for UV-C and UV-B radiations, respectively. The applied doses were obtained by varying the exposure time to the set distance, using the equation of López-Rubira et al. [22]:

$$D = (F \times t)/1000, \quad (1)$$

where D is the dose of the radiation applied (kJ/m²), F is the radiant flow (W/m²), and t is the time of exposure (in seconds).

The seeds were placed in a plastic tray (100 per species), in such a way that the surface closest to the embryo was exposed to the source of radiation, thus seeking to reduce possible interference by endosperm tissues. The biological material was introduced in an irradiation chamber with a UV-C radiation-emitting lamp (model UVM-225D, mark UVP) at a constant irradiance of 0.16 mW/cm² (equivalent to 1.6 W/m²). The same procedure was performed with a UV-B radiation lamp (model 3UV-36, 3UV Lamp) at a constant intensity of 0.15 mW/cm² and 50 cm distance from the lamp.

Treatments were set as: (a) minimum dose, which was defined as the first indication of change or modification in the seedling structure; (b) maximum dose, which was established as the one where the changes in the seedling structures were more visible, up to 80% of seeds lots; (c) lethal dose (LD50), which was marked when 50% or more of the seeds didn't show germination; and (d) a control (without UV radiation).

Table 1. Doses of UV-B and UV-C radiations with exposure times for seed germination of four species in a constant irradiance chamber.

Species	UV Radiation Type	Treatments						
		Control (T1)	Minimum Dose (T2)		Maximum Dose (T3)		Lethal Dose (T4)	
				kJ/m ²	Time (s)	kJ/m ²	Time (s)	kJ/m ²
<i>Glycine max</i>	UV-B	Without UV radiation	5.4	3600	43.2	28800	56.7	37800
	UV-C	Without UV radiation	1.44	900	43.2	27000	57.60	36000
<i>Triticum aestivum</i>	UV-B	Without UV radiation	1.35	900	8.1	5400	37.8	25200
	UV-C	Without UV radiation	2.88	1800	17.28	10800	43.2	27000
<i>Helianthus annuus</i>	UV-B	Without UV radiation	1.35	900	0.27	180	8.1	5400
	UV-C	Without UV radiation	2.88	1800	8.64	5400	43.2	27000
<i>Pinus maximartinezii</i>	UV-B	Without UV radiation	0.63	420	0.99	660	1.17	780
	UV-C	Without UV radiation	0.288	180	0.864	540	1.056	660

2.2. Standard Germination Tests

After the seed irradiation, the standard germination test was carried out, placing 100 seeds (four repetitions of 25 seeds) on a seedburo paper k-22, previously moistened with water, according to the techniques proposed by Moreno [20] and the International Seed Testing Association (ISTA) [21]. The seeds were covered with another paper, which was later rolled up, forming paper rolls which were introduced in the germination chamber at 25 ± 2 °C. Then, the quantification of the germination rate was carried out at a determined time, depending on each species, likewise the number of normal and abnormal seedlings was recorded, as well as the number of ungerminated seeds, according to Huron [23] methodology.

For *Helianthus annuus* and *Glycine max*, the initial counts were performed on the fifth day, for *Triticum aestivum* on the fourth day and for *Pinus maximartinezii* on the seventh day after planting. A second count was made, 8 days later for sunflowers, 10 days for soybeans, 7 days for wheat, and 21 days for pine, to finish the germination rate. In addition, the average length of plumule and radicle were recorded.

2.3. Accelerated Aging Test and Vigor of Seedlings

Conditions were induced to increase the rate of the seeds' physiological deterioration, using an artificial aging chamber with a temperature of 45 °C and relative humidity of 100%. Inside the chamber, a 600 ml beaker containing 250 ml of water was placed, with 200 seeds on a plastic mesh above the water level, sustained by a support inside and covered with a plastic film, according to the procedures proposed by Moreno [20] and Huron [23]. There were a total of 40 bottles in the chamber. The exposure time applied was 64 hours for soybeans, 48 hours for wheat and sunflowers, and 72 hours for pine. At the end of the exposure period of the seeds to high temperature and humidity conditions, the standard germination tests and the corresponding accelerated aging tests were carried out.

2.4. Experimental Design and Statistical Data Analysis

The data obtained from the seed germination and seedling vigor tests were analyzed with the SPSS statistical package (version 21), where a completely randomized design was applied with a bifactorial arrangement: Factor 1 consisted of the four plant species, and Factor 2 consisted of the four doses of radiation, which generated a total of 16 treatments with eight repetitions per treatment for both UV-B and UV-C radiation. The average values of the results were compared using the Tukey test [24].

3. Results

3.1. Standard Germination Test under UV-B Radiation

The averages of the variables evaluated showed a highly significant difference ($p < 0.01$), both between the species and between the treatments for the variables normal seedlings (NS), average length of plumule (ALP), and average length of radicle (ALR) (Table 2).

Table 2. ANOVA of standard germination test in four plant species under UV-B radiation.

Source of variation	Df	Vigor (%)	F _{cal}	GR	F _{cal}	NS	F _{cal}	ALP	F _{cal}	ALR	F _{cal}
Treatment	3	3.80	2.01	3.35	1.58	272.30	52.61 **	67.30	25.93 **	8.69	3.62 **
Species	3	64.34	33.98 **	61.77	29.1 **	470.28	90.87 **	121.83	46.94 **	907.62	378.68 **
T×S	9	1.21	0.64	0.98	0.46	46.57	9.00 **	17.28	6.66 **	11.62	4.85 **
S.E.		1.89		2.12		5.18		2.60		2.40	
C.V.%		5.84		6.19		15.51		21.36		10.73	

GR = germination rate; NS = normal seedlings; ALP = average length of plumule; ALR = average length of radicle; ** = highly significant ($p < 0.01$); Df = degree of freedom; CV = coefficient of variation; F_{cal} = F calculated; T×S = treatment by species; S.E. = standard error.

The vigor presented average values of 99.6% for soybeans, 98% for wheat, 87.3% for sunflowers, and 92.1% for pine. The germination rate (GR) showed statistically equal averages for soybeans and wheat, registering the values 98.8 and 98.2%, respectively, however, for sunflowers the value was 87.2%, and for pine it was 92.1%. The average for NS was higher in wheat (81.3%), followed by soybeans (54.2%), sunflowers (49.25%), and pine (49.7%). In the variables of ALP and ALR, the average values were 8.03 cm and 19.68 cm for soybeans, 7.31 cm and 15.58 cm for wheat, 9.9 cm and 15.57 cm for sunflowers, and 4.89 cm and 6.85 cm for pine (Figure 1).

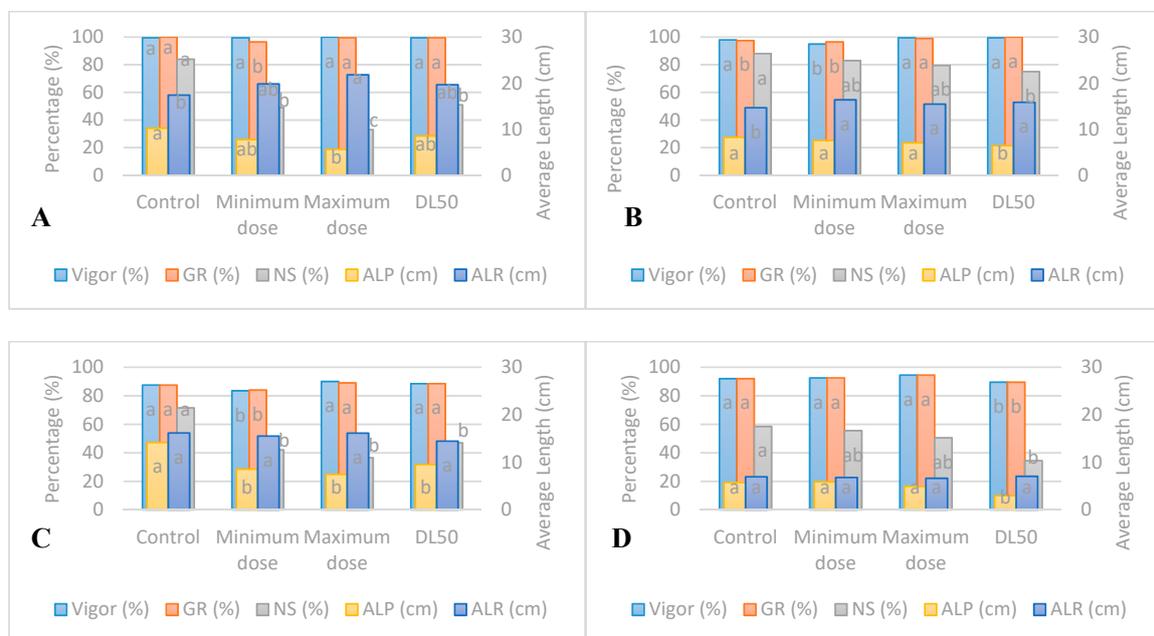


Figure 1. Standard germination tests in soy, wheat, sunflower and pine seedlings under UV-B radiation. (A). *Glycine Max*, (B). *Triticum aestivum*, (C). *Helianthus annuus*, (D). *Pinus maximartinezii*; GR = Germination rate, NS = normal seedlings, ALP = average length of plumule, and ALR = average radicle length. Different letters in columns with the same color indicate significant differences ($p < 0.05$).

3.2. Accelerated Aging Test under UV-B Radiation

For the test of accelerated aging under UV-B radiation, the ANOVA showed significant differences both between species ($p < 0.05$) and among the irradiation doses applied (Table 3).

Table 3. ANOVA of accelerated aging test in four plant species under UV-B radiation.

Source of Variation	Df	Vigor (%)	F	GR (%)	F	NS (%)	F	ALP (cm)	F	ALR (cm)	F
Treatment	3	1.84	0.62	3.13	1.14	456.75	92.81 **	134.78	91.95 **	7.36	1.54
Species	3	106.80	35.89 **	120.97	44.05 **	890.11	180.8 **	161.49	110.17 **	784.31	164.23 **
T×S	9	3.07	1.03	2.64	0.96	76.00	15.44 **	39.66	27.06 **	6.46	1.35
S.E.		2.97		2.74		4.92		1.46		4.77	
C.V.%		7.74		7.38		18.43		20.64		16.56	

GR = germination rate; NS = normal seedlings; ALP = average length of plumule; ALR = average length of radicle; ** = highly significant ($p < 0.01$); CV% = coefficient of variation; T×S = treatment by species; S.E. = standard error.

For the vigor of seedlings, the values 97.2, 93.12, 82.0, and 83.8% were registered for soybeans, wheat, sunflowers, and pines respectively, and the germination registered values of 97.3% (soybeans), 95.6% (wheat), 82.3% (sunflowers), and 83.8% (pine). UV-B radiation negatively influenced the values of normal seedlings of pine (35.5%), soybeans (36.6%), and sunflowers (40.7%), compared to wheat (79.6%). Similarly, the ALP and ALR were affected by UV-B radiation, with pine (3.1 cm and 5.6 cm) being the most affected species, compared to sunflowers (8.5 cm and 16.3 cm) and wheat (6.8 cm and 15.1 cm).

In terms of treatments, the vigor (91–98%) and germination (95–99%) values of soybeans and wheat were kept constant, however, the number of normal seedlings (NS) showed high variation (17.5–78.5% and 75–84%) for these species. The variables of ALP (2.99–10.17 cm) and ALR (14.76–16.96 cm) did not show significant differences ($p > 0.05$), depending on the treatments. In summary, treatment 2 (minimum dose) turned out to be less harmful for these two species.

Regarding sunflowers and pine, the variables vigor and germination capacity showed a variation of 80–85% and 81–89%, respectively, while the values corresponding to the number of normal seedlings ranged from 21.5% to 68% for sunflowers, and from 24% to 46.5% for pine. The ALP variables ranged from 4.54 cm to 13.83 cm for sunflowers and from 2.26 cm to 4.1 cm for pine. ALR ranged between 14.94 cm and 17.052 cm and 5.42 cm and 5.86 cm for sunflowers and pine, respectively.

From a statistical point of view, soybeans and wheat formed a single group for vigor and germination, depending on treatments (Figure 2), while sunflowers and pine formed another group, indicating significant differences between species, both for vigor ($F = 35.89, p < 0.01$) and for germination ($F = 44.05, p < 0.01$). As for ALR, soybeans, wheat, and sunflowers formed a group.

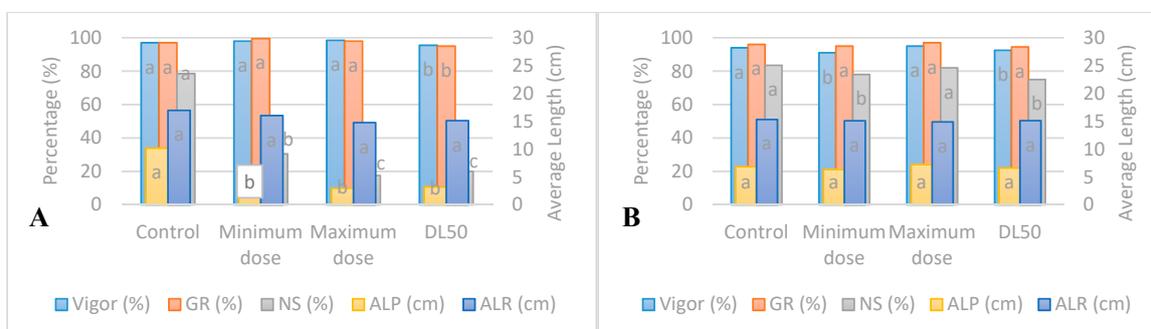


Figure 2. Cont.

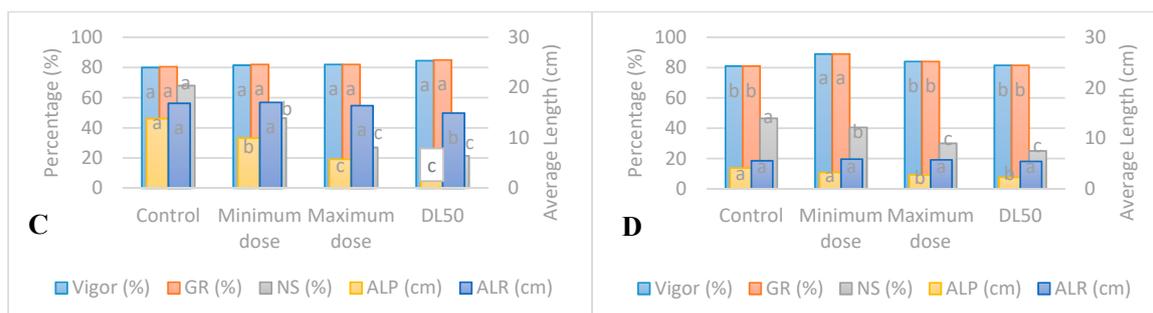


Figure 2. Accelerated aging tests in soybeans, wheat, sunflowers, and pine seedlings under UV-B radiation. (A). *Glycine Max*, (B). *Triticum aestivum*, (C). *Helianthus annuus*, (D). *Pinus maximartinezii*; GR = germination rate, NS = normal seedlings, ALP = average length of plumule, ALR = average length of radicle. Different letters in columns with same color indicate significant differences ($p < 0.05$).

3.3. Standard Germination under UV-C

The analysis of variance showed highly significant differences ($p < 0.01$), both between species and treatments (Table 4) for all the variables studied, with average values of 100%, 98.6%, 76%, and 55% of germination for soybeans, wheat, sunflowers, and pine, respectively (Figure 3). The average value for normal seedlings was higher in wheat (84%), followed by soybeans (76.5%), and finally by sunflowers (36%), and pine (25%). The plumule and radicle lengths were 13.5 cm and 19.2 cm for soybeans, 8.2 cm and 14.3 cm for wheat, 6.2 cm and 13.2 cm for sunflowers, and 2.2 cm and 3 cm for pine, respectively.

Table 4. ANOVA of standard germination test in four plant species under UV-C radiation.

Source of Variation	Df	Vigor (%)	F _{cal}	GR (%)	F _{cal}	NS	F _{cal}	ALP	F _{cal}	ALR	F _{cal}
Treatments	3	27.65	6.36 **	25.84	5.47 **	75.55	13.55 **	22.71	9.77 **	14.53	2.78 **
Species	3	913.47	210.02 **	940.64	199.2 **	1698.63	304.7 **	729.99	313.9 **	1473.10	2818 **
T×S	9	37.65	8.66 **	39.11	8.29 **	15.40	2.76 **	7.86	3.38 **	14.02	2.68 **
S.E.		4.35		4.72		5.57		2.33		5.23	
C.V.%		10.14		10.77		17.16		20.22		18.27	

GR = germination rate; NS = normal seedlings; ALP = average length of plumule; ALR = average length of radicle. ** = highly significant ($p < 0.01$); Df = degree of freedom; CV% = coefficient of variation (%); F_{cal} = F calculated, T×S = treatment by species; S.E. = standard error.

In the seeds of *Glycine max*, the vigor and germination rate were kept constant with a value of 100%, both in the treatment of maximum and minimum doses of irradiation with UV-C as well as the control. The normal seedling variable had values from 72% to 88%, with the lethal dose being the treatment which showed the most harmful effect. The radicle length values ranged from 19 cm to 20.1 cm, where no significant differences were detected ($p > 0.05$) between treatments.

The wheat presented values of 96% to 100% in vigor and germination rate, while the normal seedlings varied from 80% to 92%, and plumule length from 7.8 cm to 9 cm.

In sunflowers, the values ranged from 72% to 80% for vigor, 68% to 76% for germination, 28% to 40% for NS, and 11 cm to 16 cm for ALR, stating that the DL50 and maximum doses were the treatments that caused the most damage to the seeds of this species.

For pine, values of 52% to 72% were recorded for vigor, 72% for germination, 12% to 36% for NS, and 1 cm to 3 cm of ALP, highlighting that the DL50 and minimum doses were more harmful for these variables with irradiation UV-C.

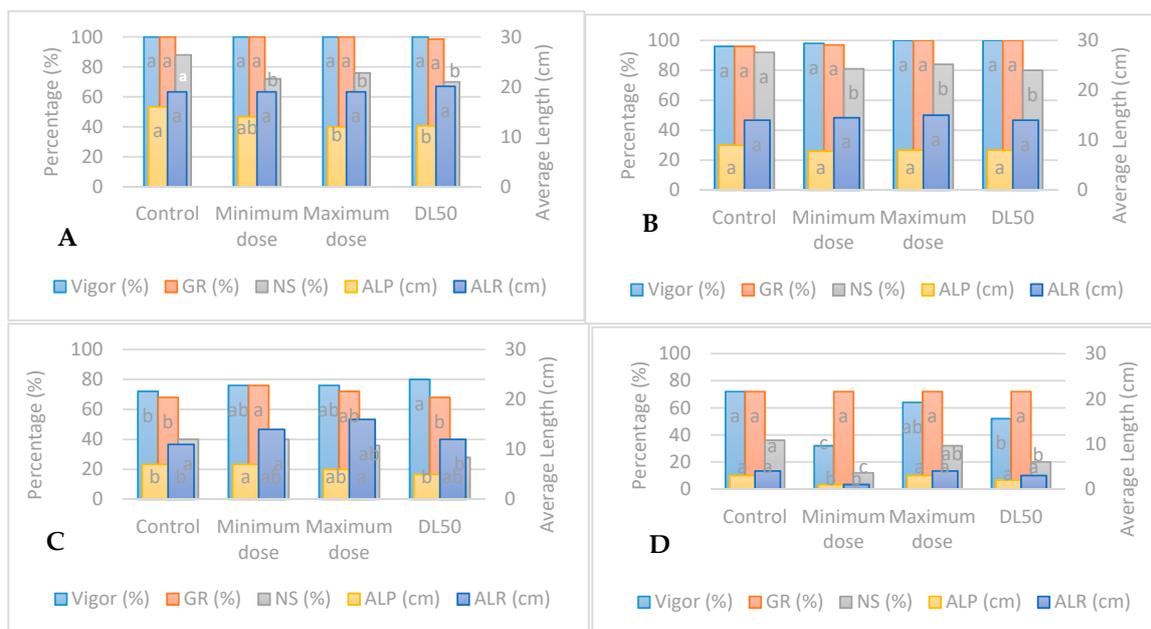


Figure 3. Standard germination tests performed on seeds of four species irradiated with UV-C at different concentrations: (A). *Glycine Max*, (B). *Triticum aestivum*, (C). *Helianthus annuus*, (D). *Pinus maximartinezii*; GR = germination rate (%), NS = normal seedlings, ALP = average length of plumule; ALR = average length of radicle. Different letters in columns with the same color indicate significant differences ($p < 0.05$).

3.4. Accelerated Aging Test under UV-C Radiation

The analysis of variance showed significant differences ($p < 0.01$) both between species and between treatments for the evaluated variables (except in NS, ALP, and ALR) (Table 5). The vigor presented average values of 98.8%, 86.3%, 76.6%, and 36.2% for soybeans, wheat, sunflowers, and pine, respectively (Figure 4). For germination, average values of 97%, 88%, 75.3%, and 36% were recorded for soybeans, wheat, sunflowers, and pine, respectively. For the ALP and ALR variables, the average values oscillated from 3.625 cm to 5.615 cm and 16.187 cm to 18.65 cm for soybeans and wheat, respectively, with no significant differences ($F = 0.30$; $p > 0.05$). The NS recorded the ranges 3.5–8.5% and 23.5–60.5% for sunflowers and pine, respectively. The ALP for sunflowers and pine varied from 0.711 cm to 1.495 cm and 2.275 cm to 6.77 cm, respectively. As for the ALR, the values from 12.652 cm to 14.39 cm, 2.425 cm to 5.255 cm, and 4.467 cm to 4.99 cm were obtained for wheat, sunflowers and pine, respectively.

Table 5. ANOVA of accelerated aging test in four plant species under UV-C radiation.

Source of variation	Df	Vigor %	F _{cal}	GR %	F _{cal}	NS	F _{cal}	ALP	F _{cal}	ALR	F _{cal}
Treatment	3	30.20	3.47 **	26.09	3.32 *	127.65	2.87 *	278609	4.95 **	10.76	0.30
Species	3	3907.88	448.52 **	3831.69	488.05 **	0	0	0.37	0.07	1080.58	30.62 **
T×S	9	121.25	13.92 **	127.24	16.21 **	54.51	1.22	14.17	2.52	2.05	0.06
S.E.		8.7		7.85		44.55		5.63		35.29	
C.V.%		15.84		15.11		73.90		59.00		61.07	

GR = germination rate, NS = normal seedlings, ALP = average length of plumule, ALR = average length of radicle. ** = highly significant ($p < 0.01$); Df = degree of freedom; CV% = coefficient of variation, F_{cal} = F calculated, T×S = treatment by species; S.E. = standard error.

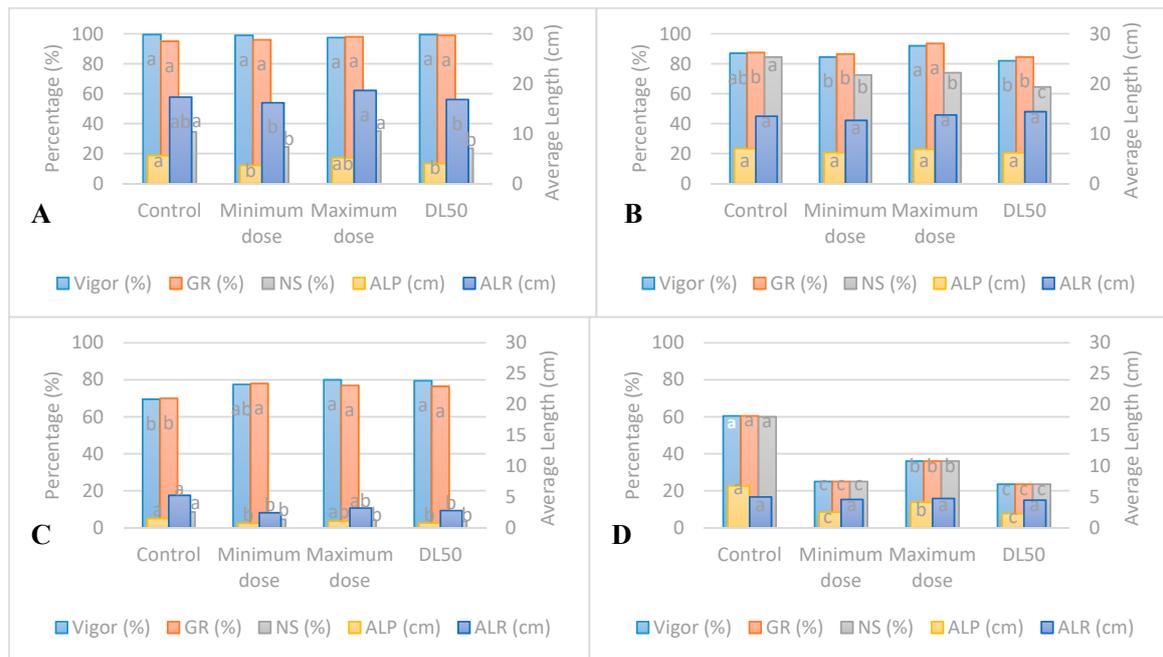


Figure 4. Accelerated aging test in seedlings of four species irradiated with UV-C at different concentrations. (A). *Glycine Max*, (B). *Triticum aestivum*, (C). *Helianthus annuus*, (D). *Pinus maximartinezii*; GR = germination rate, NS = normal seedlings, ALP = average length of plumule, ALR = average length of radicle. Different letters in columns indicate significant differences ($p < 0.05$).

3.5. Responses of Seedlings to UV-B and UV-C Radiations

The changes caused by the effect of UV-B and UV-C irradiation on the soybean seeds were manifested in the hypocotyl, presenting necrotic damage, curvature under the cotyledons, and interlacing of the cotyledons that restricted the emergence of the epicotyl, as well as cracks or divisions in the radicle (Figure 5).



Figure 5. Soybean seedlings from seeds irradiated with UV-B and UV-C (observed in stereoscope at 10X magnification).

The alterations shown in the wheat seedlings due to UV-C and UV-B irradiation in their seeds were coleoptile fissures from the base of the root, as well as the torsion of the plumule and the strangulation of the mesocotyl (Figure 6).



Figure 6. Wheat seedlings from seeds irradiated with UV-B and UV-C (observed in stereoscope at 10X (a) and 40X (b,c)).

The sunflower seedlings suffered different malformations derived from UV-C irradiation, including kinking, cracking, and hypocotyl curvature, in addition to deformation of their tissues, forming excrescences and ruptures, which caused asymmetric growths (Figure 7).

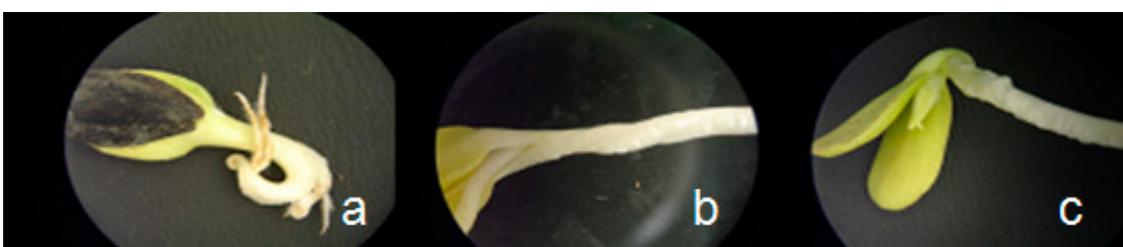


Figure 7. Sunflower seedlings from seeds irradiated with UV-B and UV-C (observed in stereoscope at 10X (a) and 40X (b,c)).

Finally, in pine, the alterations that presented the seedlings were fissures from the radicle base and curvature of the hypocotyl, in addition to the deformation of their tissues, forming different lesions that induced the inhibition of hypocotyl elongation as well as its curvature (Figure 8).



Figure 8. Pine seedlings from seeds irradiated with UV-B and UV-C.

4. Discussion

4.1. Standard Germination of Seeds Irradiated with UV-B

The seeds of soybeans and wheat presented minimal sensitivity to UV-B radiation, which increases as the dose of radiation applied increases. UV-B inhibited the growth and nutrient metabolism of soybeans during germination [25], causing the ALP and ALR to decrease proportionally to the amount of UV-B applied. This agrees with Shinklea et al. [26] and Lizana et al. [27], who mentioned that the response to the short wavelength UV-B (280 nm and 300 nm) induces the inhibition of hypocotyl elongation and curvature, and a positive phototropism in both cucumber and *Arabidopsis* seeds. In the same way, Mazza et al. [28] and Selezneva [29] have detected the responses to current levels of UV-B solar energy, such as reductions in leaf surface expansion and biomass accumulation rate, and also the reduction of growth.

The high-dose UV-B treatments could cause irreversible damage of cellular macromolecules, such as proteins, nucleic acids, and lipids, due to the absorption of the energy rich radiation [30]. When the

soybeans germinated under the UV-B radiation, it was shown that the cell volume became smaller, the cell shape tended to be irregular, and the cell space became greater. This might be because UV-B radiation increased cell permeability, caused loss of membrane integrity, and resulted in water released to the intercellular space [25].

Sunflowers were the species most affected by UV-B radiation compared to the other three species evaluated, causing abiotic stress due to high radiation and heat. Nawkar et al. [5] pointed out that the specific photoreceptors of UV-B light at low fluence induce a protective response and at high intensity stress to the plants. As the response to such voltage is independent of the photoreceptor, plants irradiated with UV-B can activate the path of cell death and biotic stresses.

Pine, similarly to soybeans, showed minimal sensitivity to UV-B radiation. The increase in the applied radiation dose interferes with the growth of the plumule (ALP) and the radicle (ALR), which decreased proportionally to the amount of UV-B applied. These results agree with those reported by Jansen et al. [31] and Tural et al. [32], who mentioned that UV-B radiation interferes with the growth, development, photosynthesis, flowering, pollination, and transpiration of plants.

4.2. Standard Germination of Seeds Irradiated with UV-C

The soybean and wheat seeds showed similar behaviors to the effect of the radiation dose. However, pine and sunflower seeds presented physiologically different behavior, where sunflowers showed greater sensitivity to UV-C radiation. This could be related to what was reported by Kanash and Savin [33], who mentioned that the wheat plant shows tolerance to UV-C radiation, maintaining its productivity with applications for 7 hours at doses of 100–250 kJ/m²/day. These differences could be attributed to the difference in the structure of the seeds, since soybean and wheat seeds are light-colored and have a thin tegument compared to sunflower and pine seeds. Similarly, Rivera et al. [15] mentioned that the susceptibility of plant tissue to irradiation differs significantly among varieties, physiological states, composition, and epidermis thickness of the plant structure. Plants have been found to activate enzymatic and non-enzymatic antioxidant systems to counter different stresses on treating with low levels of UV B and UV C [34].

Sarghein et al. [7] and Aguirrezabal et al. [35] pointed out that the effects of UV radiation vary between species and cultivars, showing reductions in plant growth, photosynthetic activity, and flowering. It should be mentioned that wheat and soy plants show good tolerance at high irradiance, with the optimum dose corresponding to maximum dose (43.20 kJ/m²/day). This has been reported in UV-C irradiated groundnut seeds, where there was an increase in seed germination, seedling vigor, biomass production, and all seedling growth parameters with increasing periods of UV-C exposure up to 60 min, as compared to seed controls [36].

The sunflower and pine species were the most affected by UV-C radiation, decreasing their vigor and germinative power, with a high degree of deterioration in the pine seed. These results corroborate with those described by Reglinski et al. [37], who demonstrated that the use of UV-C irradiation induces resistance to fungal infection in a conifer species (*Pinus radiata*). Similarly, Torres et al. [38] and Cerero-Hernández [39] indicated that the percentage of normal plants in sunflowers was reduced when exposure to UV-C irradiation increased from 5 min to 60 min, which caused acute physiological stress, which led to a reduction in growth.

4.3. Accelerated Aging of Seedlings from Seeds Irradiated with UV-B

Sunflowers and pine showed greater sensitivity to the effects of UV-B radiation, while soybeans and wheat presented physiologically different behavior. These differences could be attributed to their different contents of oils, proteins, and other compounds in their seeds structures. According to Tripathi et al. [40], oil content and seed quality parameters in sunflowers—such as total sugar, protein, amino acids, and oil content—were more affected under combined exposure of UV-B and O₃.

Both sunflowers and pine showed high sensitivity, due to the high concentration of lipids in their seeds, which would facilitate the oxidation and deterioration of the plasma membrane. Therefore, the

response of the evaluated variables is very low, due to the stress caused by the aging test being in the limit of germination allowed by the ISTA [21] to be considered as high-quality seed, contrary to what happened in UV-C radiation. Based on the variables evaluated, the best responses were obtained with the application of UV-B under the minimum dose (T2) for sunflowers (1.35 kJ/m²/day) and for pine (0.63 kJ/m²/day). In these species, the reduction in growth was measured through the expansion of the hypocotyl and radicle, a consequence of the effects of the applied radiation, which agrees with that obtained by Cechin et al. [41], who confirm that UV-B radiation affects the rate and duration of cell division and elongation.

For soybeans, the minimum dose (5.4 kJ/m²/day) presented similar responses to the control. UV-B radiation induces a wide variety of responses in plants, including changes in stem elongation and leaf morphology. These results coincide with those of Boccalandro et al. [42], who pointed out that UV-B radiation induces various phenotypic responses in plants.

Wheat did not present statistical difference between its treatments. The average values obtained were above the accepted limit to be considered good quality seed and the small decreases were due to the reduction of the biomass. According to Lizana et al. [27], the features that are most related to the loss of performance are increases in UV-B radiation in the early stages of development. Proteins and lipids are direct targets of UV-B radiation. Since proteins strongly absorb ~280 nm or higher wavelengths, UV-B radiation can affect aromatic amino acids such as tyrosine, phenylalanine, and tryptophan [5].

4.4. Accelerated Aging of Seedlings from Seeds Irradiated with UV-C

The results obtained in this test showed the same trend as the standard germination test, where sunflowers and pine presented greater sensitivity to the combination of UV-C radiation and the stress caused by high humidity and temperatures, due to their greater lipid and protein content. Concerning this, Beena et al. [43] mentioned that at high temperatures (70 °C), changes occur in the metabolism of soybeans, specifically in the protein bands, since soybeans contain proteins and are rich in lipids. In the case of soybeans, treatment 3 (maximum dose = 43.2 kJ/m²/day) presented similar responses to the control. These results are consistent with those mentioned by Minuzzi [44] and Rai et al. [45], who indicated that the lipid and protein content of soybeans are highly influenced by humidity and temperature. The average values of vigor and germination, depending on treatments, for wheat are within the accepted limit to be considered good quality seed. According to Belgin et al. [46], UV-C radiation together with abiotic factors induces biological stress in plants, as well as defense mechanisms of plant tissues with the consequent production of phytoalexins. The results on the production of normal plants could be a reflection of modifications in the mineral composition, content of starches, and specific metabolites such as flavonoids, which are known to be induced by UV-C radiation.

A high sensitivity was found in sunflower and pine seeds, due to the concentration of lipids in their seeds, which would facilitate the oxidation and deterioration of the plasma membrane. Therefore, the response of the evaluated variables is very low, due to the stress caused by the aging test. These results agree with those obtained by Mpoloka [47], who pointed out that UV-C radiation is responsible for alterations in plants, including damage to DNA, affecting physiological processes. Moreover, Deng et al. [48] reported that high temperatures and UV-C irradiation differentially affect the biosynthesis of specific stilbenes, including resveratrol and piceatannol, in *Gnetum parvifolium* stems and roots.

However, different results have been reported in other species, showing, for example, that the use of UV-C and fluorescent light are advantageous for controlling soft rot in potato plants without adversely affecting sprouting [49]. Seed treatments with low doses of UV-C (3.6 kJ/m²/day) were used to elicit host resistance to black rot in cabbage (*Brassica oleracea* L.), improving the quality and growth response of cabbages under greenhouse conditions [19]. Similarly, lettuce seeds treated by exposure to 0.82 and 3.42 kJ/m²/day UV-C doses were able to mitigate the impact of excessive salinity, possibly as result of the enhanced free radical scavenging activity detected in their leaf tissues, where a

dose-dependent response occurs—seedlings derived from seeds treated with the lowest UV-C dose showed higher tolerance to salinity conditions [50].

5. Conclusions

The good tolerance to the UV radiation tested allowed the determination of the adequate doses, which correspond to the maximum dose (T3) of UV-C radiation (43.20 kJ/m²/day) for the germination and vigor of soybeans, wheat, and pine, and the minimum dose (T2) (2.88 kJ/m²/day) for sunflowers. Regarding UV-B radiation for germination and accelerated aging tests, the minimum doses used in this study were the adequate dose, which presented the right responses and the least damage to the seedlings of the four species, both for the germination and vigor.

Sunflowers and pine showed higher sensitivities than wheat and soybeans under UV-B radiation in the aging test. The harmful effects manifested in the soybean seedlings irradiated with UV-B and UV-C were the curvatures and necrotic damage in the hypocotyl and cross-linking of the cotyledons, which restricted the emergence of the epicotyl, as well as fissures in the radicle. In the sunflowers, curls, fissures, and curvatures of the hypocotyl were presented, as well as malformations and ruptures of their tissues, which caused asymmetric growths. In the pine, cracks appeared from the base of the radicle and curvature of the hypocotyl. In the wheat seedlings, torsions and fissures of the plumule were presented from the base of the root. The wheat presented minimal alterations, and thus it can be considered a species relatively tolerant to UV-C and UV-B radiations.

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References

1. Kerr, J.B.; Mcelroy, C.T. Evidence for large upward trends of Ultraviolet-B radiation linked to ozone depletion. *Science* **1993**, *262*, 1032–1034. [[CrossRef](#)] [[PubMed](#)]
2. Winter, T.R.; Rostas, M. Ambient ultraviolet radiation induces protective responses in soybean but does not attenuate indirect defense. *Environ. Pollut.* **2008**, *155*, 290–297. [[CrossRef](#)]
3. Frohnmeyer, H.; Staiger, D. Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiol.* **2003**, *133*, 1420–1428. [[CrossRef](#)] [[PubMed](#)]
4. Pombo, M. Irradiación de frutillas con UV-C: Efecto sobre la síntesis de proteínas, degradación de la pared celular y mecanismos de defensa. Doctoral Thesis, Universidad Nacional de San Martín (UNSAM), Laboratorio de Bioquímica y Fisiología de la Maduración y Senescencia (UB-4) IIB-INTECH (Chascomús), San Martín, Argentina, 2009.
5. Nawkar, G.M.; Maibam, P.; Parque, J.H.; Sahi, V.P.; Lee, S.Y.; Kang, C.H. UV-Induced Cell Death in Plants. *Int. J. Mol. Sci.* **2013**, *14*, 1608–1628. [[CrossRef](#)]
6. Robson, T.M.; Klem, K.; Urban, O.; Jansen, M.A.K. Re-interpreting plant morphological responses to UV-B radiation. *Plant Cell Environ.* **2015**, *38*, 856–866. [[CrossRef](#)]
7. Sarghein, S.H.; Carapetian, J.; Khara, J. The Effects of UV Radiation on Some Structural and Ultrastructural Parameters in Pepper (*Capsicum longum* A.DC.). *Turk. J. Biol.* **2011**, *35*, 69–77.
8. Yagura, T.; Makita, K.; Yamamoto, H.; Menck, C.F.; Schuch, A.P. Biological Sensors for Solar Ultraviolet Radiation. *Sensors* **2011**, *11*, 4277–4294. [[CrossRef](#)] [[PubMed](#)]
9. Ruiz, L.G.A.; Qüesta, A.G.; Rodríguez, S.C. Efecto de luz UV-C sobre las propiedades antioxidantes y calidad sensorial de repollo mínimamente procesado. *Rev. Iber. Tecnología Postcosecha* **2010**, *11*, 101–108.
10. Sgroppo, S.C.; Sosa, C.A. Zapallo anco (*Cucurbita moschata*, D.) fresco cortado tratado con luz UV-C. *Facena* **2009**, *25*, 7–19.
11. Fonseca, J. Efecto de la luz UV-C en la calidad de hortalizas. *Revista Productores de Hortalizas* **2009**, 2009.

12. Rastogi, R.P.; Kumar, A.R.; Tyagi, M.B.; Sinha, R.P. Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair. *J. Nucleic Acids* **2010**, *2010*, 32. [[CrossRef](#)] [[PubMed](#)]
13. Luckey, T.D. *Hormesis with Ionizing Radiation*; CRC press: Boca Raton, FL, USA, 1980; p. 222.
14. Rastogi, R.P.; Richa; Kumar, A.; Tyagi, M.B.; Sinha, R.P. Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair. *J. Nucleic Acids* **2010**, *2010*, 32. [[CrossRef](#)]
15. Rivera, P.D.M.; Gardea, B.M.; Martínez, T.A.; Rivera, D.M.; González, A. Efectos Bioquímicos postcosecha de la irradiación UV-C en frutas y hortalizas. *Rev. Fitotec. Méx.* **2007**, *30*, 361–372.
16. Häder, D.P.; Koumar, H.D.; Smith, R.C.; Worrest, R.C. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochem. Photobiol. Sci.* **2007**, *6*, 267–285. [[CrossRef](#)]
17. Castronuovo, D.; Sofo, A.; Lovelli, S.; Candido, V.; Scopa, A. Effects of UV-C radiation on common dandelion and purple coneflower: First results. *Int. J. Plant Biol.* **2017**, *8*, 7255. [[CrossRef](#)]
18. Kataria, S.; Guruprasad, K.N. Solar UV-B and UVA/B exclusion effects on intraspecific variations in crop growth and yield of wheat varieties. *Field Crops Res.* **2012**, *125*, 8–13. [[CrossRef](#)]
19. Brown, J.E.; Lu, T.Y.; Stevens, C.; Khan, V.A.; Lu, J.Y.; Wilson, C.L.; Collins, D.J.; Wilson, M.A.; Igwegbe, E.C.K.; Chalutz, E.; et al. The effect of low dose ultraviolet light-C seed treatment on induced resistance in cabbage to black rot (*Xanthomonas campestris* pv. *campestris*). *Crop Prot.* **2001**, *20*, 873–883. [[CrossRef](#)]
20. Moreno, E.M. *Análisis Físico y Fisiológico de Semillas Agrícolas*, 3rd ed.; UNAM, Ciudad Universitaria: México, D.F., Mexico, 1996; p. 393.
21. International Seed Testing Association (ISTA). *International Rules for Seed Testing 1999*, 2nd ed.; Seed Science and Technology 27, Supplement of vigor test methods, ISTA: Zurich, Switzerland, 1999; p. 117.
22. López, R.V.; Artés, H.F.D.A.; Artés, C.F. Evaluación de la calidad de granadas tratadas con UV-C y almacenadas en atmósfera controlada. V Congreso iberoamericano de tecnología postcosecha y agroexportaciones. 2007, pp. 137–145. Available online: https://www.researchgate.net/publication/36720755_Evaluacion_de_la_calidad_de_granadas_tratadas_con_UV-C_y_almacenadas_en_atmosfera_controlada (accessed on 15 November 2018).
23. Huron. Foro del Parque Natural de las Sierras de Cazorla, Segura y las Villas. Reproducción de semillas de las principales especies de pinos del parque. February 2009. Available online: www.cazorlaturismo.com (accessed on 15 November 2018).
24. Zar, J.H. *Biostatistical Analysis*, 5th ed.; Prentice-Hall, Inc.: Saddle River, NJ, USA, 2010; p. 947.
25. Ma, M.; Wang, P.; Yang, R.; Gu, Z. Effects of UV-B radiation on the isoflavone accumulation and physiological-biochemical changes of soybean during germination: Physiological-biochemical change of germinated soybean induced by UV-B. *Food Chem.* **2018**, *250*, 259–267. [[CrossRef](#)]
26. Shinkle, J.R.; Atkins, A.K.; Humphrey, E.E.; Rodgers, C.W.; Wheeler, S.L.; Barnes, P.W. Growth and morphological responses to different UV wavebands in cucumber (*Cucumis sativum*) and other dicotyledonous seedlings. *Physiol. Plant.* **2004**, *120*, 240–248. [[CrossRef](#)]
27. Lizana, X.C.; Hess, S.; Calderini, D.F. Crop phenology modifies wheat responses to increased UV-B radiation. *Agric. For. Meteorol.* **2009**, *149*, 1964–1974. [[CrossRef](#)]
28. Mazza, C.A.; Boccacandro, H.E.; Giordano, C.V.; Ballaré, C.L. Significación funcional y de inducción de la radiación solar ultravioleta de protectores solares que absorben en los cultivos de soja cultivadas en el campo. *Plant Physiol.* **2000**, *122*, 117–126. [[CrossRef](#)] [[PubMed](#)]
29. Selezneva, E.M.; Goncharova, L.I.; Tikhonov, V.N. The effects of UV-irradiation of barley plants on the morphophysiological parameters and productivity of the offsprings. *Radiats Biol. Radioecol.* **2008**, *48*, 487–492. [[PubMed](#)]
30. Jiao, J.; Gai, Q.Y.; Wang, W.; Luo, M.; Gu, C.B.; Fu, Y.J.; Ma, W. Ultraviolet radiation-elicited enhancement of isoflavonoid accumulation, biosynthetic gene expression, and antioxidant activity in *Astragalus membranaceus* hairy root cultures. *J. Agric. Food Chem.* **2015**, *63*, 8216–8224. [[CrossRef](#)]
31. Jansen, M.A.K. Ultraviolet-B radiation effects on plants: Induction of morphogenic responses. *Physiol. Plant. A.* **2002**, *116*, 423–429. [[CrossRef](#)]
32. Turtola, S.; Sallas, L.; Holopainen, J.K.; Julkunen-Tiitto, R.; Kainulainen, P. La exposición prolongada a la radiación UV-B mejorada no tiene efectos significativos sobre el crecimiento o compuestos secundarios de pino Silvestre y exterior crecido y Noruega plántulas de piceas. *Environ. Contam.* **2006**, *144*, 166–171.
33. Kanash, E.V.; Savin, V.N. The sensitivity of agricultural plants to short-term UV-stress. *Kosm. Biol. Aviakosm. Med.* **1991**, *25*, 18–20. [[PubMed](#)]

34. Thomas, T.T.D.; Puthur, J.T. UV radiation priming: A means of amplifying the inherent potential for abiotic stress tolerance in crop plants. *Environ. Exp. Bot.* **2017**, *138*, 57–66. [CrossRef]
35. Aguirrezabal, L.A.N.; Orioli, G.A.; Hernandez, L.; Pereyra, V.R.; Mirave, J.P. *Girasol: Aspectos Fisiológicos que Determinan el Rendimiento*; Unidad Integrada Balcarce (ISBN N°950-9853-71-2). Offset Vega: Buenos Aires, Argentina, 1996; p. 127.
36. Neelamegam, R.; Sutha, T. UV-C irradiation effect on seed germination, seedling growth and productivity of groundnut (*Arachis hypogaea* L.). *Int. J. Curr. Microbiol. Appl. Sci.* **2015**, *4*, 430–443.
37. Reglinski, T.; Taylor, J.T.; Chee, A.A.; Northcott, G.; Spiers, M. Biochemical responses to Ultraviolet-C radiation and methyl jasmonate in *Pinus radiata* seedlings that accompany induced resistance to *Diplodia pinea*. *Plant Pathol.* **2012**, *62*, 851–858. [CrossRef]
38. Torres, M.; Frutos, G.; Duran, J.M. Sunflower seed deterioration from exposure to U.V.-C radiation. *Environ. Exp. Bot.* **1991**, *31*, 201–207. [CrossRef]
39. Cerero, H.N. Girasol, situación actual, mundial y nacional. CONASIPRO. Available online: http://www.oleaginosas.org/art_237.shtml (accessed on 7 January 2019).
40. Tripathi, R.; Rai, K.; Singh, S.; Agrawal, M.; Agrawal, S.B. Role of supplemental UV-B in changing the level of ozone toxicity in two cultivars of sunflower: Growth, seed yield and oil quality. *Ecotoxicology* **2019**, 1–17. [CrossRef] [PubMed]
41. Cechin, I.; De Fátima, F.T.; Ligia Dokkedal, A. Crecimiento y respuestas fisiológicas de las plantas de girasol expuestas a la radiación ultravioleta-B. Universidad Federal de Santa Maria. Centro de Ciencias Rurais. *Cienc. Rural* **2007**, *37*.
42. Boccalandro, H.E.; Mazza, C.A.; Ballaré, C.L. La radiación ultravioleta B mejora la respuesta photomorphogenic fitocromo-B mediada en *Arabidopsis*. *Plant Physiol.* **2001**, *126*, 780–788. [CrossRef]
43. Beena, A.K.; Jayaram, K.M. Electrophoretic variations in seed protein profile of green pea (*Pisum Sativum* L.) and soybean [*Glycine max* (L.) MERR.] seedlings during early stages of germination under heat-stress. *Legume Res.* **2010**, *33*, 171–177.
44. Minuzzi, A.; Mora, F.; Sedrez Rangel, M.A.; Scapim, C.A. Características Fisiológicas, Contenido de Aceite y Proteína en Genotipos de Soya, Evaluadas en Diferentes Sitios y Épocas de Cosecha, Brasil. *Agric. Téc.* **2007**, *67*, 353–361. [CrossRef]
45. Rai, R.; Meena, R.P.; Smita, S.S.; Shukla, A.; Rai, S.K.; Pandey-Rai, S. UV-B and UV-C pre-treatments induce physiological changes and artemisinin biosynthesis in *Artemisia annua* L. an antimalarial plant. *J. Photochem Photobiol B.* **2011**, *105*, 216–225. [CrossRef]
46. Erdoğan, S.B.; Ekiz, H.I. Effect of Ultraviolet and Far Infrared Radiation on Microbial Decontamination and Quality of Cumin Seeds. *J. Food Sci.* **2011**, *76*, M284–M292. [CrossRef]
47. Mpoloka, S.W. Effects of prolonged UV-B exposure in plants. *Afr. J. Biotechnol.* **2008**, *7*, 4874–4883. [CrossRef]
48. Deng, N.; Liu, C.; Chang, E.; Ji, J.; Yao, X.; Yue, J.; Bartish, I.V.; Chen, L.; Jiang, Z.; Shi, S. High temperature and UV-C treatments affect stilbenoid accumulation and related gene expression levels in *Gnetum parvifolium*. *Electron. J. Biotechnol.* **2017**, *25*, 43–49. [CrossRef]
49. Rocha, A.B.; Honório, S.L.; Messias, C.L.; Otón, M.; Gómez, P.A. Effect of UV-C radiation and fluorescent light to control postharvest soft rot in potato seed tubers. *Sci. Hortic.* **2015**, *181*, 174–181. [CrossRef]
50. Ouhibi, C.; Attia, H.; Rebah, F.; Msilini, N.; Chebbi, M.; Aarrouf, J.; Urban, L.; Lachaal, M. Salt stress mitigation by seed priming with UV-C in lettuce plants, growth, antioxidant activity and phenolic compounds. *Plant Phys. Biochem.* **2014**, *83*, 126–133. [CrossRef]

