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1 **CHARACTERIZATION OF BEAN SEEDS, GERMINATION AND FENOLIC**
2 **COMPOUNDS OF SEEDLINGS BY UV-C RADIATION**

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1 **CHARACTERIZATION OF BEAN SEEDS, GERMINATION AND FENOLIC**
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6 **ABSTRACT**

7 We quantitatively evaluate the effects of UV-C radiation on bean seeds Oti (*Phaseolus vulgaris*), their
8 germination and phenolic compounds of seedlings, by means of photoacoustic spectroscopy and scanning
9 electron microscopy. It is shown that: *i*) The photoacoustic signal produced by the bean seeds exposed to UV-
10 C radiation during 15 minutes, reduces from 0.24 to 0.10 mV for the wavelengths of 295 and 340 nm, as a result
11 of their degradation. This significant reduction of 58.3% decreases, but keeps over 50% for other spectral UV
12 wavelengths within the interval (270-400 nm). *ii*) As the UV-C radiation time increases from 2 to 15 min, the
13 morphological changes at the surface of the bean seeds exhibits micro-holes and detachment of the cotyledon
14 sclereids. *iii*) There are no significant statistical differences in the variables of germination, dry weight and
15 seedling length, for exposure times up to 15 minutes. *iv*). The phenolic acids and flavonoids of the bean
16 seedlings of seeds treated by UV-C for different exposure times (0, 2, 5, 10 and 15 min) display significant
17 statistical differences ($P \leq 0.05$) in both roots and foliage. For both the roots and foliage, the highest
18 concentration of phenolic acids is observed at 10 and 15 minutes, while for root flavonoids, it appears at 5 and
19 15 minutes.

20
21 **Key words:** bean seeds, secondary metabolites, UV-C radiation, photoacoustic spectroscopy.
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1 INTRODUCTION

2 One of the challenges of the humanity is to improve food production in quantity and quality for a current
3 population over 7 billion and projected for 2050 of 9 billion (FAO 2009a;2009b; Baulcombe et al. 2009;
4 Godfray et al. 2010; Araujo et al. 2016), reducing the environmental impact (Edmondson et al. 2014; Hernández
5 et al. 2016). Therefore, it is necessary to reduce the use of chemical products in the production process of food
6 (Altieri 2014). For this purpose, in this century has been intensified research into alternative physical methods
7 that are called "emerging technologies" in response to the needs of the modern world (Rifna et al. 2019). Which
8 could help stop the destructive processes of the soil, the environment and protect the production of food and
9 consequently the health and quality of life of the population (Vasilevski 2003; Hernández et al. 2016).

10
11 In this way, there are various physical methods, used in different phenological stages, by them could interest
12 the pre-sowing stage (Hernández et al. 2006; 2007;2009; Aladjadjiyan 2008). The pre-sowing methods allow a
13 conditioning of the seed; producing beneficial effects in early stages of growth (Thomas and Pothur 2016;
14 Aladjadjiyan 2012), as in germination, which conditions the vigor of the plants and could be the basis for
15 intensification of crop production (Mariz-Ponte et al. 2018). Physical methods for seed invigoration have
16 advantages over conventional treatments based on chemical substances, e.g. reduce the use of fertilizers,
17 fungicides and pesticides, etc., thus could decreasing pollution (Rifna et al. 2019; Aladjadjiyan 2012). Within
18 these emerging physical methods, are those based on technology laser, led, magnetic and electric fields, non-
19 thermal plasma, ultrasound, ionizing radiation (gamma rays and x-rays), microwave, ozone, magnetically
20 treated water, electrolyzed oxidizing water, and Ultraviolet (UV) radiation, among others (Vasilevsky 2003;
21 Marinković et al. 2008; Hernandez et al. 2010;2016; Aladjadjiyan 2012; Occhipinti 2014; Maffei 2014; Araujo
22 et al. 2016; Thomas and Pothur 2016; Rifna et al. 2019).

23
24 Ultraviolet radiation to low levels for treatment of seeds is an ecologically safe method, then, an
25 environmentally-friendly alternative to improve plant productivity, yield quality and as well as to induce
26 tolerance of plants to various biotic and abiotic stresses (Forges et al. 2018; Thomas and Puthur, 2016;
27 Ehsanpour and Razavizadeh 2005). UV light is an electromagnetic radiation of wavelengths shorter than the
28 visible region, but longer than X-rays (Shetta and Areaf 2009). UV radiation has been classified as: UV-A (320–
29 400 nm), called black light, UV-B (280–320 nm), called medium wave and UV-C (200–280 nm), called short
30 wave or germicidal (Katerova and Todorova 2009; Shetta and Areaf 2009). The damaging effect of UV
31 radiation increases towards shorter wavelengths: UV-A is less effective than UV-B, while UV-C photons are
32 highly energetic, and quickly create high levels of damage (Katerova and Todorova 2009; Barta et al. 2004;
33 Stapleton 1992).

34
35 The UV light continuous or pulsed has been studied to have a broad application in the food and agriculture
36 industry (Elmnasser et al. 2007; Gómez-López et al. 2007; Alexieva et al. 2001; Mukhopadhyay and
37 Ramaswamy 2012). In particular, in the agriculture, the UV-C radiation non-ionizing continuous have shown

1 to stimulate positive impact on seed health, germination (G), and seedling vigor (SV), also has been applied in
2 different fruit, vegetable and legume crops, among others (Sukthavornthum et al. 2018; Dawar 2013). In
3 legumes, most applications have focused on the health-related effects of seed, seedlings, seedling roots and / or
4 crops (fungal control and sterilizing effects) (Dawar 2013; Siddiqui et al. 2011), as well as the effects at the
5 level of seedlings and plants radiated with UV-C (Da Silva 2019; Kara 2013) and to a lesser degree,
6 physiological effects (G and SV) and / or of secondary metabolites caused by UV-C light when is used as pre-
7 sowing treatment to the seed and as well the possible degradation produced in it. Among the legumes, most
8 consumed in various countries of the world, e.g. Africa, India, Latin America and Mexico is the bean (Chávez-
9 Mendoza and Sánchez 2017).

10 In this way, the present investigation experimental aims are: a) to investigate the degradation of bean seed
11 treated with UV-C light using Photoacoustic spectroscopy (PAS), as well as to evaluate their morphological
12 changes by means of scanning electron microscopy, b) evaluate the physiological quality of the seed (through
13 the germination, seedling length and dry weight) and the phenolic compounds (phenolic acids and flavonoids)
14 of the seedlings grown from seed treated with UV-C radiation prior to sowing.

15
16 Globally 800 million people are malnourished (Broughton et al. 2003), being one problems in Mexico and other
17 countries in the world. Beans are considered important because they are a source of protein, fiber, iron, complex
18 carbohydrates, minerals and vitamins (Grela et al. 2017). In addition, characteristics of Nutraceuticals
19 compounds have been identified as polyphenols, flavonoids and anthocyanins (Lin et al. 2008). In such a way
20 that it is a healthy option for the consumption of the human population. For this reason, it is one of the crops
21 that requires improving its production.

22 **MATERIALS AND METHODS**

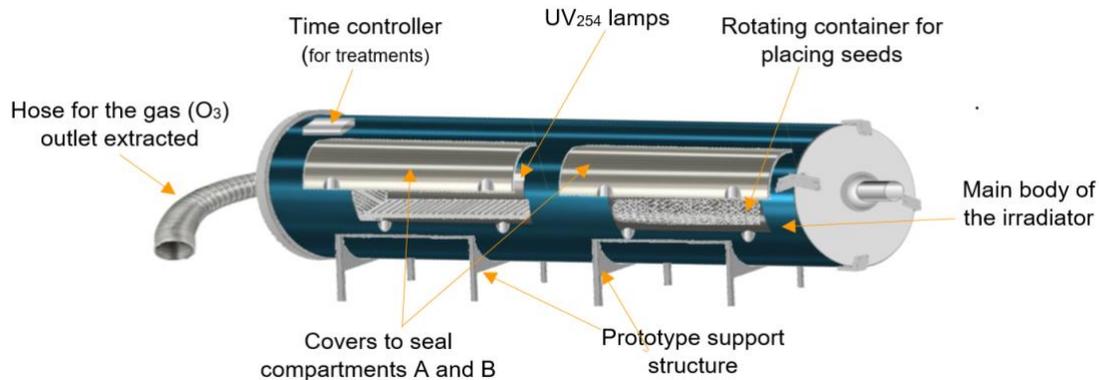
23 **Biological material**

24 In the present research project, bean seeds “Oti” (*Phaseolus vulgaris* L.) of the cycle productive vegetation
25 period 2017 were used. These have been provided by the Mexican Genetic Quality control assurance institute-
26 Seed Program of the “Instituto de Recursos Genéticos y Productividad del Colegio de Postgraduados“-
27 (IREGEP). The seed lot was standardized and 100-seed weight averages were 31.84 g and length, width and
28 thickness (l_s), were about 11.508 ± 0.56 , 7.6 ± 0.39 and 5.24 ± 0.5 mm, respectively. The measurements were
29 realized by digital micrometer (Mitutoyo).

31 **Treatment of UV-C radiation**

32 After homogenizing the bean seed, it was treated pre-sowing, with UV-C radiator system (UV-C/RS-
33 Homemade). The system consisted of an arrangement of 4 lamps (UV-C, 254 nm) accommodated on the top
34 and bottom of a cylindrical stainless steel base (Figure 1). The lamps when turned on emits light towards a
35 rotating wire-mesh container. Bean seeds were positioned in this container. Several radiation times were applied

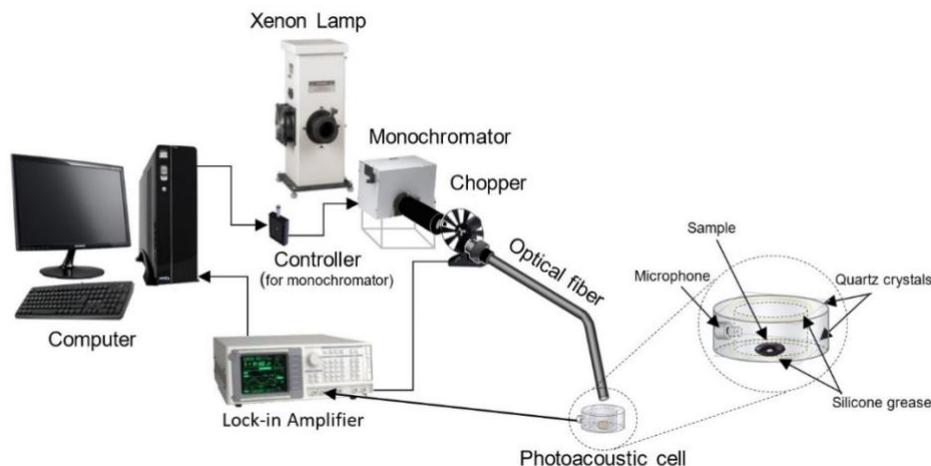
1 to the seeds: 0, 2, 5, 10 and 15 min, programmed through a timer. The light intensity measured by the UV-
 2 C/254 measuring equipment was of $700 \mu\text{w}/\text{cm}^2$.



3
 4 **Figure 1** UV-C radiator system

5
 6 **PAS EXPERIMENTAL SETUP**

7 The experimental setup of PAS consists of a Xenon lamp used as the light excitation source, a monochromator
 8 applied to obtain a monochromatic light beam, and a mechanical chopper employed to modulate the light beam
 9 at the fixed frequency $f = 17 \text{ Hz}$, as shown in Fig. 2. The modulated beam was focused onto an optical fiber to
 10 guide it to the Photoacoustic (PA) cell, whose signal was then detected by an electret microphone (Photoacoustic
 11 sensor) connected to the cell through a fine channel (1 mm diameter). The PA signal was amplified by means
 12 of a Lock-in amplifier (EG&G, Mod. 5210) before being recorded on a personal computer, as a function of
 13 beam wavelength. Experiments were performed for wavelengths between 275 to 700 nm and by placing the
 14 samples into the PA cell with a diameter of 6 mm. The samples of bean seeds of each treatment UV-C were
 15 prepared on a circular form and diameter of 5 mm, such that they do not obstruct the microphone sensor and
 16 can be measurements when located inside the Photoacoustic cell.



17
 18 **Figure 2** Scheme of the PAS experimental setup used in our experiments.

1 ***Calculation of absorption coefficient and optical penetration length.***

2 The determination of the absorption coefficient (β) and optical penetration length (l_β) of the bean seed were
 3 determined from the equation of Poulet et al. (1980), as has been indicated by Hernández et al. (2011a; 2011b)
 4 for the characterization of agricultural plant seed. It is possible to observe in equation (1), β is a function of the
 5 PA signal of amplitude (q) obtained by the PAS

6
$$\beta = \frac{q}{\mu_s} \frac{q + \sqrt{2 - q^2}}{1 - q^2}, \quad (1)$$

7 Which it was used after verification of the condition thermal thickness of the seed: $l/\mu_s \gg 1$, where $\mu_s = \sqrt{\alpha/\pi f}$
 8 is the thermal diffusion length for the frequency of 17 Hz; finally, α and l_s the sample thermal diffusivity and
 9 thickness, respectively.

10 The q = PA signal of normalized amplitude, α = thermal diffusivity. In this case, the value corresponding to
 11 simple starch was used = 4.44×10^{-3} cm²/s (Sanchez et al. 2014). Finally, the optical penetration length l_β was
 12 calculated from the inverse of β , being $l_\beta = 1/\beta$.

13 ***Scanning electron microscopy***

14 The morphological changes of the beans seeds, control and treated with the exposition times of UV-C radiation
 15 (2, 5, 10 and 15 min) were analyzed with a scanning electron microscope (JEOL JSM-6010LA, Tokyo, Japan)
 16 under high vacuum. The whole bean seeds were cut in half, placed in an aluminum sample holder, and fixing
 17 them with a carbon tape. The samples were covered with gold nanoparticles. The micrographs were obtained
 18 from the surface of the whole bean seeds at 5000 x. In the bean cut in half, the test and starches were analyzed
 19 at 1000x. The conditions of analysis in the equipment were 15 kV of voltage of acceleration of electrons.

20

21 ***Germination test***

22 Germination test was established under a randomized complete block design, with five repetitions and 20 seeds
 23 as an experimental unit. Seeds after irradiation were seeded on absorbent paper (double 24x23.2cm) moistened;
 24 with 40 ml of distilled water.

25 The germination stage was considered finished 12 days after the experiment was established. The temperature
 26 and humidity conditions corresponded to an average temperature of 24.9 °C and 26% humidity. At the end of
 27 the germination, fresh weight of seedlings was obtained, and subsequently their dry weight. Roots and foliage
 28 were separated to obtain biomass dry weight after drying in an oven (Felisa® Model 293) at 45 °C for 48 h.
 29 After drying the bean seedling organs were milled and sieved (U.S.A. Standard testing sieve NO°50 300
 30 micrometer 0.117 inches). Then were perform the analysis by HPLC.

31

1 **Analysis by HPLC**

2 Extracts were obtained with 50 mg of dry and pulverized material in 1 mL of methanol of HPLC grade (Sigma-
3 Aldrich number 36860) at 80%, incubated for 20 min in an ultrasound bath (BRANSON at Smithkline company
4 50/60 Hz, model B-220, USA) (Meneses Reyes et al. 2008). The crude extracts were centrifuged at 731 g
5 (Centrifuge 5804 eppendorf, model 5804) for 10 min (Irakli and Ekateriniadou, 2018). Supernatants were
6 filtered with 25 mm diameter acrodiscs with nylon membrane and 0.45 mm pore size (Titan). These extracts
7 were injected immediately for the analysis by HPLC of phenolic acids and flavonoids. For the analysis of free
8 flavonoids additionally a hydrolysis was made exposing the extracts at 70 °C for one hour. Each extract was
9 realized in duplicate.

10 **Phenolic acids**

11 The samples were analyzed in a Hewlett Packard® chromatograph mod. 1100 provided with diode array
12 detector and an Agilent Technologies automatic injector model 1200. The column was a Nautilus 125 x 4.0 mm
13 d. i., 5 µm. The mobile phase was distilled water adjusted to pH 2.5 with trifluoroacetic acid and acetonitrile. The
14 analysis was by gradient: T1 0.10 min (85% A) (15% B); T2 20 min (65% A) (35% B) and T3 23 min (65%
15 A), (35% B), $\lambda = 280$ nm, column temperature 30 °C and flow of 1 mL min⁻¹ (Bilia et al. 2001). Calibration
16 curves were performed for the galic acid, chlorogenic acid, syringic acid, vanillic acid, p-hydroxybenzoic acid,
17 caffeic acid, ferulic acid and p-coumaric acid standards (Sigma-Aldrich).

18 **Flavonoids**

19 **Flavonoid glycosides**

20 A Nucleosil 100-5 C-18 Nautilus 125 x 4.0 mm d. i., 5 µm column was used. The mobile phase was distilled
21 water adjusted to pH 3 with H₃PO₄ (A) and acetonitrile (B). The analysis was by gradient: T1 0.10 min (88%
22 A) (12% B); T2 10 min (82% A) (18% B), T3 15 min (82% A), (18% B), T4 30 min (55% A), (45% B) and T5
23 32 min (0% A), (10% B), $\lambda = 254, 316, 365$ nm, column temperature 30 °C and flow of 1.3 mL min⁻¹; $\lambda = 350$
24 nm and the column temperature was 30 °C (Bilia et al. 2001). Calibration curve was performed for apigenin-7-
25 0-glucoside, phlorizin, rutin and naringin (Sigma-Aldrich).

26 **Free flavonoids**

27 A Hypersil ODS HP column of 125 mm length and 4 mm internal diameter, 5 µm particle size was used. The
28 mobile phase was distilled water adjusted to pH 2.5 with trifluoroacetic acid (A) and acetonitrile (B). The
29 analysis was by gradient: T1 0.10 min (85% A) (15% B); T2 20 min (65% A) (35% B) and T3 25 min (65%
30 A), (35% B), $\lambda = 254, 316$ and 365 nm, Column temperature 30 °C and flow of 1 mL min⁻¹. Calibration curves
31 were performed for standards: rutin, phlorizine, quercetin, naringin, naringenin, fletetin, galangin (Sigma-
32 Aldrich).

33 The interpolations of all the extracts were calculated with ChemStation software © Agilent Technologies, Inc.
34 2004.

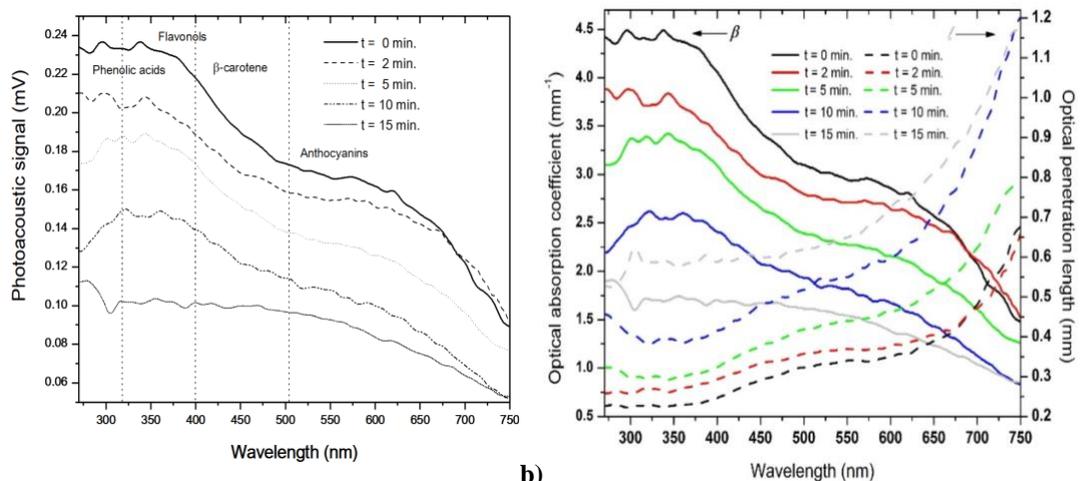
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1 RESULTS AND DISCUSSION

2 By the application of photoacoustic spectroscopy and scanning electron microscopy for evaluate the effects of
 3 UV-C radiation on bean seeds was found than there were differences between the control and treated samples.
 4 According to PAS, there is a degradation of the seed due to the radiation, there were differences between the
 5 photoacoustic spectra obtained for the control seeds ($T_0= 0$ min) and for each one of the radiation treatments
 6 applied ($T_1 = 2$, $T_2 = 5$, $T_3 = 10$ and $T_4 = 15$ min) prior to sowing.

7 Figure 3a shows the optical absorption spectra where it is noted that the level of the photoacoustic signal
 8 decreases with the increase of the radiation time of the bean seed (O_{ti}). Note that the control seeds presented
 9 the highest photoacoustic signal intensity level when compared with the spectra obtained from the seeds treated
 10 with UV-C.

11 The higher changes were obtained in the phenolic and flavonoid acids absorption band, at the lowest
 12 wavelength, mainly in the absorption band of 270-450 nm. It is observed that after 15 min of UV-C treatment
 13 of the bean seed, the maximum absorption region tends to disappear. The photoacoustic signal shows the
 14 maximum absorption in the UV region (A, B and C) according to the range of the photoacoustic absorption
 15 spectrum obtained (270-750 nm).



16 a)

b)

17 **Figure 3** Spectra of optical absorption obtained by PAS in control seeds (without UV-C radiation) and seeds
 18 treated with UV-C at 2, 5, 10 and 15 min: a) Photoacoustic signal b) Optical absorption coefficient (β) and
 19 optical penetration length (l_{β}) as a function of wavelength for seed samples.

20 Table 1 (Section a) shows the averages values of the photoacoustic signal (PA) obtained by PAS at different
 21 wavelengths for each one of the seed groups treated with UV-C. It was found, significant statistically differences
 22 $p \leq 0.05$ in the signal PA value at the different wavelength of the seeds treated, from $\lambda_{280} - \lambda_{430}$, that covers
 23 the ultraviolet region (280-400 nm); note that from λ_{445} , there were no significant statistical differences.

1 It is observed that the absorption region of 280-430 nm, significant statistical differences were found in the
 2 wavelengths of 280, 295, 310, 325, 340, 355, 370, 385, 400, 415 and 430 nm. Being the maximum decrease in
 3 the level of photoacoustic signal at the wavelengths of 295 and 340 nm, there being a percentage of decrease of
 4 58.33 %.

5 In the range of $\lambda_{280} - \lambda_{430}$, the percentage of decrease in the value of maximum intensity of photoacoustic signal
 6 (control seeds) and the lowest value obtained in seeds T₄ (15 min) varied between 52 and 58%. The higher
 7 decrease in the photoacoustic signal was found in the UV region range. For wavelength values above 430 nm,
 8 a variation between the maximum and minimum photoacoustic signal value between 40 and 50% was obtained.
 9 In this way, significant changes in the photoacoustic absorption spectra are related, due to the changes in the
 10 bean seed produced by the different radiation times applied in the bean seed.

11 Other authors evaluating milk powder by photoacoustic spectroscopy found changes in optical absorption
 12 spectra due to different times of UV-C radiation. They related the optical absorption spectra with changes in
 13 their oxidation levels (Dóka et al. 2000). In this research, is demonstrated that the content of secondary
 14 metabolites such as phenolic acids and flavonoids are modified in seedlings from seed radiated by UV-C light.
 15 Then, there is a decrease in the level of intensity of the photoacoustic signal. Since there is a relationship
 16 between the level of the photoacoustic signal and the presence of secondary metabolites in the bean seeds,
 17 which in bean seeds has been reported by other authors (Reynoso et al., 2007). If the photoacoustic signal level
 18 is increased, it is because there are more chromophores associated at those wavelengths. The chromophores
 19 would be the secondary metabolites present in the bean seed, which are affected by UV-C radiation.

20 Figure 3b represents the spectra of the optical absorption coefficient and the optical penetration length. It is
 21 observed that the highest values of β correspond to the spectra with the highest level of PA signal; it was the
 22 spectrum corresponding to the control seeds (without radiation). The bean seeds were thermally thick, which
 23 means that $l_s/\mu_s = l_s/(\omega/\pi f)^{1/2} = 577 \gg 1$. By using the eq. 1, were calculated β and l_β . Figure 3b shows the
 24 optical absorption coefficient (β) and optical penetration length (l_β), as a function of the wavelength. The sample
 25 average thickness $l_s = 5.24 \text{ mm} \pm 0.5$, according to the measurements of 100 seeds made by digital micrometer.
 26 It is possible to observe that bean seeds are optically opaque in the 270 to 750 nm range, which means that $l_\beta <$
 27 l_s for all wavelengths and treatments applied to the seeds in the different radiation times UV-C (0, 2, 5, 10, and
 28 15 min). Fulfilling this condition since the values of the optical penetration length were lower than 1.2 mm.
 29 Also, it is observed that the optical penetration length increases as the radiation time of the seed increases for
 30 the entire range of wavelength evaluated (270 - 750 nm).

31

32 The l_β values decrease towards the ultraviolet region; the control seeds had the lowest optical penetration values.
 33 It was found that to the higher degradation of the bean seed, the penetration length increases in the UV-A region
 34 (320-400 nm) and with the tendency to decrease toward the UV-B (280-320 nm) and UV-C (200-280) nm. The
 35 portion of the UV-C spectrum that is observed in Figure 3b, is the corresponding to 270-280 nm.

1 The value of the optical penetration length of the control seeds and those radiated at the different times (T_1 , T_2 ,
2 T_3 and T_4) varied from 0.22-0.65 mm, at wavelength 270 nm, corresponding to the UV-C range. According to
3 the trend of the curve, these values tend to decrease towards the wavelength value of 254 nm (maximum peak
4 of UV-C lamps). The value in the control seeds is lower and the higher penetration value corresponds to the
5 case of the seeds treated with UV-C for 15 min, which is the maximum time of exposure of the seed to radiation
6 in this study; also, showed the higher degradation to this time of radiation (T_4). It should be mentioned that the
7 thermal diffusion length ($\mu_s = 0.009$ mm) is smaller than the optical penetration length.

8
9 In this investigation it was found that the degradation of the bean seed did not cause a decrease in the
10 physiological quality of the seed; expressed through germination, length and dry weight of seedlings. Variables
11 which, when compared with control, did not find significant statistical differences ($p \leq 0.05$) (Table 1-Section
12 b). Although there is a tendency to increase and then decrease in the germination of seeds irradiated by 10 and
13 15 min, respectively. The results show that PAS is sensitive to detect changes in bean seed, observed as a color
14 degradation in the seed itself. These changes were verified in this investigation, through the scanning electron
15 microscopy.

16

17

18

19

1 **Table 1** Averages of parameters obtained of seeds and seedlings of bean a) level of photoacoustic signal obtained by PAS at different wavelengths b)
 2 germination variables of seedlings from treated seed.

3

Treatment (min)	a. Photoacoustic signal at different wavelengths													b. Variables of seed germination			
	λ_{280}	λ_{295}	λ_{310}	λ_{325}	λ_{340}	λ_{355}	λ_{370}	λ_{385}	λ_{400}	λ_{415}	λ_{430}	λ_{445}	λ_{460}	λ_{475}	G	TW	L
0) Control																	
(0)	0.23a	0.24a	0.23a	0.23a	0.24a	0.23a	0.23a	0.22a	0.21a	0.20a	0.19a	0.19a	0.18a	0.18a	86a	2.41a	9.48a
1) 2	0.21b	0.21b	0.21ba	0.20ba	0.21ba	0.20a	0.20a	0.19a	0.19ba	0.18ba	0.17ba	0.17ba	0.16ba	0.16ba	80a	2.51a	9.9a
2) 5	0.17c	0.18c	0.19b	0.19bc	0.19bc	0.19ba	0.18ba	0.18ba	0.17ba	0.16ba	0.15bac	0.15bac	0.14bac	0.14ba	75a	1.86a	9.6a
3) 10	0.13d	0.14d	0.15c	0.15c	0.15c	0.15bc	0.15bc	0.14bc	0.14bc	0.13bc	0.13bc	0.12bc	0.12bc	0.11ba	87a	1.95a	10.3a
4) 15	0.11e	0.10e	0.10d	0.10d	0.10d	0.10c	0.10c	0.09c	0.10c	0.10c	0.09c	0.09c	0.09c	0.09b	79a	2.25a	11.06a
LSD (0.05%)	0.02	0.03	0.03	0.03	0.04	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	20.9	0.464	0.608
Media	0.171	0.175	0.173	0.174	0.175	0.174	0.172	0.168	0.164	0.158	0.152	0.147	0.144	0.141	81.4	2.20	28.91
Significance	0.0002**	0.0007**	0.0015**	0.0033**	0.0045**	0.0087**	0.01*	0.01*	0.01*	0.03*	0.05*	0.06ns	0.08ns	0.10ns	0.7ns	0.3*	0.01*
C.V. (%)	3.68	5.26	6.55	7.61	8.62	9.78	10.29	10.49	11.55	12.8	14.48	15.38	16.16	16.93	19.16	27.64	0.48
R ²	0.99	0.99	0.98	0.97	0.97	0.96	0.96	0.96	0.95	0.94	0.93	0.92	0.91	0.90	0.25	0.68	0.98

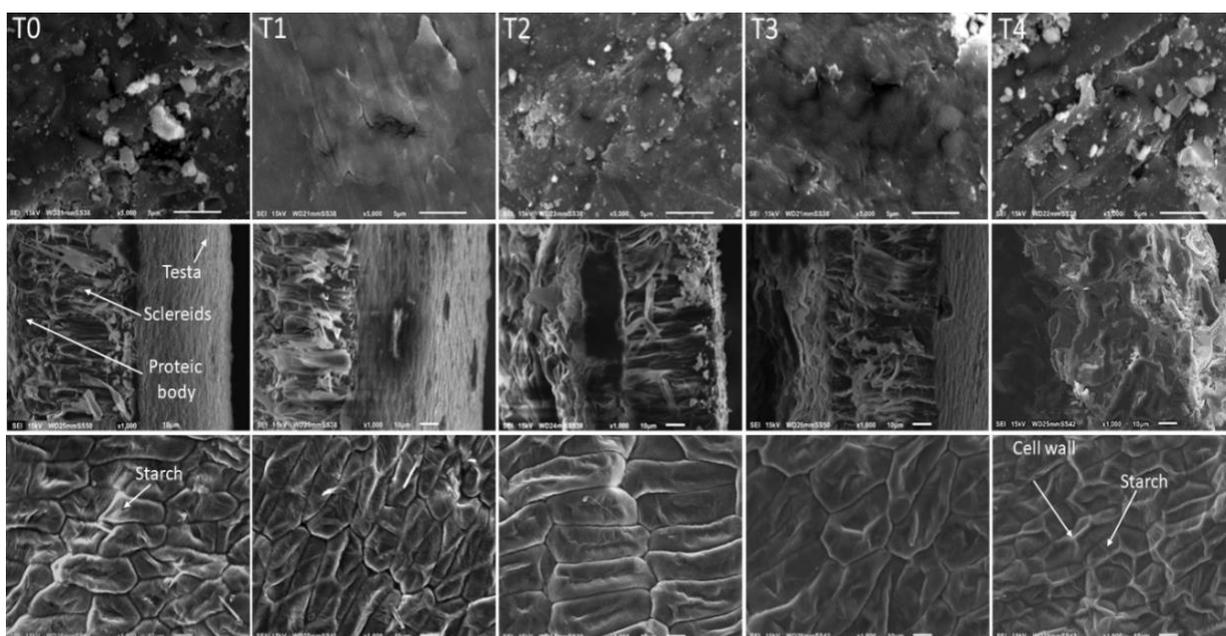
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5

6 Means with the same letter in a column are statistically equal (LSD (Lows significant differences) , 0.05); λ –wavelength, (λ_{280} , λ_{295} , λ_{310} λ_{475})
 7 represent the photoacoustic signal at the wavelengths of 280, 295, 310, 325, 340, 355, 445, 460 and 475 nm. TW: Total weight, G: Germination percentage
 8 and L: seedling length.

1 *Analysis of micrographs*

2 Figure 4 shows micrographs of control bean seed ($T_0 = 0$ min) and exposed with UV-C light at different
 3 radiation times ($T_1 = 2$, $T_2 = 5$, $T_3 = 10$ and $T_4 = 15$ min), taken at 5000x from the surface of beans (first row)
 4 and 1000x in half of the bean seed (second and third row). In the image of the surface of the bean seed (first
 5 row) it is observed that the treatment with UV-C at different times affected significantly, because there is
 6 evidence of surface rupture notoriously observed in $T_3 = 10$ and $T_4 = 15$ min. In the second row it is observed
 7 that for the control sample (T_0), the cell wall (sclereids), mainly cellulose, hemicellulose and lignin are found
 8 attached to the testa and body of the cotyledon. As the dose of UV-C increases, the presence of micro-holes in
 9 the testa, and detachment of the sclereids of the cotyledon is evident. In the T_4 treatment, changes of structural
 10 conformation are clearly shown. Regarding the change of the starches (third row), when applying higher
 11 radiation treatments to the seed (T_3 and T_4), the starch granules are compacted, such that the cell wall of the
 12 cotyledon swells.



13

14 **Figure 4** Micrographs of control bean seed ($T_0 = 0$ min) and exposed with UVC light at different radiation
 15 times ($T_1 = 2$, $T_2 = 5$, $T_3 = 10$ and $T_4 = 15$ min),

16 Torres (1985) mentions that the cell wall of the cotyledon is mainly composed of cellulose, hemicellulose and
 17 pectic substances, which bind to most cells and provide the physical resistance of the tissue. Figure 4 could
 18 assume that the network that holds the starches radiated at the highest dose contains mostly pectic substances
 19 because it shows a gel texture, more flexible than the cell wall of the “testa”. Singh et al. (2013) found that the
 20 stability of cell walls components and the rearrangement of starch granules in cells can affect the free access of
 21 amylolytic enzymes during digestion (when beans are used for consumption), since the starch granules enclosed
 22 within the intact cells are compacted. Hussain *et al.* (2014), reported that by applying gamma radiation to bean
 23 starch the proportions of rapidly digestible starch were increased.

1 Likewise, Hyun-Jung et al. (2014) and Gani et al. (2012) showed that by applying gamma radiation the
2 degradation of amylose and amylopectin is induced, which alters the morphological, crystalline and
3 physicochemical properties of starch. Starches in their native form are sometimes not functional for some
4 processes. However, when applying UV-C radiation, the bean starches and the cell wall of the cotyledon
5 undergo changes, as was demonstrated in the present investigation. These changes, as mentioned by other
6 authors, could modify their functional properties, which would benefit the food industry as an additive. The
7 advantage of applying UV-C could be in which does not induce a significant increase in temperature, minimum
8 preparation of the sample, without dependence on some type of catalysis (Farkas, 1998), as well as a low cost.

9
10 For the condition of bean as a seed and not as grain of consumption, UV-C radiation has been reported with
11 stimulating effects on germination, both stimulant and inhibitory effects (Urban et al., 2016). As the case
12 described by Lazim and Nasur (2017) who treated sorghum seed (*Sorghum bicolor* L.) with UV-C for different
13 times (0, 30 and 60 min) did not find significant effects on germination percentage and the length of the seedling
14 roots. While showed a significantly negative impact on speed germination, seedling length and the number of
15 leaves. Similar results in the length of the seedlings germinated were found in black beans by (Guajardo-Flores
16 et al., 2014), who did not find significant statistical differences in the length of the seedlings; however, changes
17 were found in the speed of germination.

18 In the present investigation, the length of the seedlings varied in a range of 9.48 to 11.06 cm after 11 days of
19 germination; under the conditions in which the experiment was elaborated and it did not have statistical
20 differences. In this way the physiological quality is not modified, within the radiation time interval used in this
21 investigation. It is possible to note (Table 1b) that the smallest and the maximum length of the seedlings were
22 from control seed and radiated for 15 min.

23
24 The emergent physical methods have been reported as pre-sowing treatments harmless for the environment; but
25 these treatments must be applied with suitable parameters in order to improve the seed vigour and ensure better
26 plant development at later stages (Hernández et al. 2009). In this way is necessary to explore the optimal
27 radiation parameters: exposure time, the radiation intensity, the power of the light source, the radiation regimes,
28 etc. Likewise, the conditions and location of the seeds: the separation distance of them respect to the light, the
29 placement (on moving or fixed), the container on which it is placed, etc. It is important use the appropriate
30 treatment and conditions to produce the expected beneficial result.

31
32 In the present investigation, there were no significant differences between the treatments applied, but there was
33 a tendency to reduce the germination at 15 min. Other authors with a longer exposure time (up to 180 min)
34 found a stimulating effect on wheat seeds, improving germination (Rupiasih and Vidyasagar 2016). Other
35 studies also indicated a tendency to decrease and increase the percentage of germination, according to the times
36 of exposure to UV-C radiation. Neelamegam and Sutha (2015), found a decrease in germination and then an

1 increase at 5 and 60 min of exposure in groundnut seed (*Arachis hypogaea* L.) by UV-C, respectively at 10
2 days of germination.

3
4 Depending on the genotype and characteristics of the seed, the effect will be produced. In the present
5 investigation, it is observed that germination tends to decrease (T₂) and then to present a slight increase (T₃)
6 and then decrease again (T₄). In experiments carried out with sunflower seeds by Torres *et al.* (1991) it was
7 reported that the UV-C radiation between the time of 5-60 min reduced the percentage of normal seedlings.
8 Other authors have found that depending on the genotype of red bean seeds are the effects obtained on
9 germination (Peykarestan and Seify 2012).

10
11 According to Solecka (1997), exposure of plant tissues to UVC increases the concentration of reactive oxygen
12 species (ROS), which induces seed germination (Guajardo-Flores *et al.* 2014). UV-C irradiation treatment on
13 agricultural seeds has generally been reported promoted the seed germination. In experiments carried out on
14 Mung bean (*Vigna radiata*) seeds, they reported an increase in germination at times of exposure to UV-C
15 radiation of 2, 4 and 6 h (Hamid and Jawaid 2011). Although there were no significant differences in
16 germination in this investigation, a tendency to be modified by UV-C was found depending on the time of
17 exposure to radiation to this type of bean seed used.

18
19 In the case of the dry weight of the seedlings from the radiated seed and control (without radiation) (Table 1b),
20 it was found that the highest weight was obtained in the control seedlings and those radiated at 2 min. While
21 the lowest weight was for seedlings from seed radiated for 5 min (T₂).

22
23 In a separate way the weight of root and foliage; it was found that at two minutes the foliage weight tends to
24 increase, with a tendency to decrease at 5 min and then increase again at 10 min. In the case of the weight of
25 the roots, a slight tendency to decrease was obtained as the pre-sowing UV-C radiation time increases. Also,
26 other authors have indicated a modification of the weight of the components of seedlings (root and foliage).
27 Siddiqui *et al.* (2011) observed increment in shoot weight when seeds of groundnut (*Arachis hypogaea* L.) and
28 mung bean (*Vigna radiata* L.) were treated with UV-C for 10, 15, 30 and 60 min. Biomass as a measure of
29 growth makes it possible to know the distribution of dry matter among the various organs, as well as to estimate
30 the magnitude of the demand for assimilates (Hunt 1990). In the different times exposed to UV-C radiation
31 there were significant differences with respect to the dry weight accumulated in roots and foliage (Figure 5).

32
33
34
35

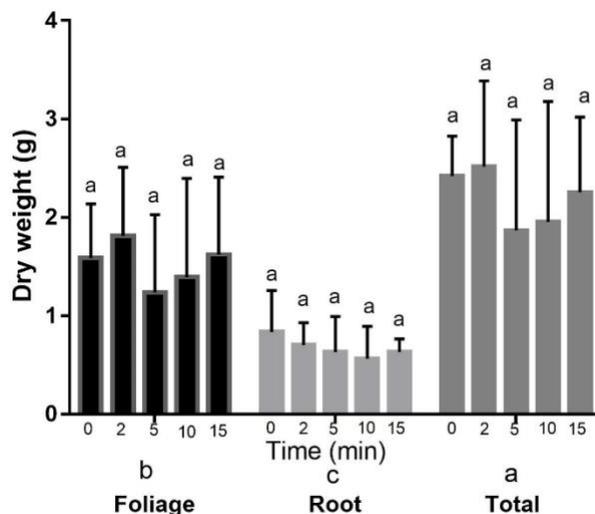


Figure 5. Cumulative dry weight of seed bean seedlings exposed to different times of shortwave ultraviolet radiation. Different letters mean that there are significant differences according to the average comparisons (Tukey $\alpha=0.05$). Time refers to different minutes of UV-C irradiation (T_0 , T_1 , T_2 , T_3 and T_4).

Other applications of UV-C radiation on agricultural seeds before sowing, is the improvement of sanitary quality, due to its sterilizing and antifungal effects (Dawar 2013; Ferreira 2011; Santos 2013; Siddiqui et al. 2011; Rodriguez et al. 2012). UV-C radiation has been used in the control of fungal growth of castor bean seeds (Dawar 2013). In this way it is important to define the appropriate radiation times because UV-C radiation degrades the seed and could modify (positive, null or negative) their physiological quality variables.

Phenolic compounds

The phenolic acids identified in the seedling from bean seeds treated pre-sowing by UVC and control (without radiation) were similar between root and foliage: gallic, chlorogenic, syringic, vanillic, p-hydroxybenzoic, caffeic, ferulic, p-cumaric; to exception of the ferulic acid which was not identified in foliage.

The phenolic acid most abundant in seedling from seed control (without radiation) was chlorogenic (18.7 $\mu\text{g/mL}$), followed by p-cumaric (2.8554 $\mu\text{g/mL}$) and caffeic (2.72 $\mu\text{g/mL}$). In the case of foliage, the most abundant were caffeic acid (78.27 $\mu\text{g/mL}$), followed by chlorogenic (18.71 $\mu\text{g/mL}$).

The phenolic acids of the bean (*Oti*) seedlings from of seed treated by UV-C for different exposure times (0, 2, 5, 10 and 15 min) showed significant statistical differences ($P \leq 0.05$) in both roots and foliage. There were significant differences according to the treatment in p-hydroxybenzoic, syringic, caffeic and ferulic of the roots (Table 2). The highest concentrations of p-hydroxybenzoic acid (2.26 $\mu\text{g/mL}$), syringic acid (1.61 $\mu\text{g/mL}$) were

1 radiated for 15 min produced seedlings with higher concentration levels of: chlorogenic acid (23.74 µg/mL),
2 p-cumaric (7.8 µg/mL) and caffeic (6.75 µg/mL). Studies carried out by other authors have coincided that the
3 application of UV radiation can induce the accumulation of phenolic compounds in legume seedlings. Shetty
4 et al. (2002) exposing seeds of faba beans (*Vicia faba*) to UV-C light for 2, 5 and 10 h and germinated for 8
5 days in the dark indicated an increase in nutraceutically-relevant phenolic metabolites with enhanced
6 antioxidant activity in response to UV stress.

7
8 In our results there was modification of phenolic acids, which in general, increased their concentration with the
9 increase of exposure times to UV-C radiation; without stress condition. *Aboul et al.* (2014), evidenced that the
10 Green bean seed treatment with UV-C improved plant growth under stressful and non-stressful conditions.
11 Growth variables were modified through exposure times of 30 and 60 minutes; *e.g.* the authors found
12 significantly enhanced shoot fresh weight of grown plants by 33.9 and 82.5%, respectively. In other studies,
13 applying UV-C in fruit epidermal tissue has been shown that UV light induces the accumulation of UV
14 absorbing flavonoids and other phenolics compounds (Strack 1997).

15 16 **Flavonoids** 17

18 Regarding to phenolic compounds of the flavonoid type, it can be observed (Table 3), that the flavonoids
19 identified in roots of control and radiated seedlings were: rutin, phorizine, quercetin, naringenin, floretin,
20 galangin, naringin, hespiridin, apigenin-7-0-glucoside. In foliage there were similars flavonoids identified in
21 the roots, except the phloretin that was not identified. The flavonoid most abundant in this type of bean seed
22 (Oti) was Phlorizin (460 µg/mL).

23
24 Flavonoids of the seedlings from of seed treated by UV-C for different exposure times (0, 2, 5, 10 and 15 min)
25 showed significant statistical differences ($P \leq 0.05$) in both root and foliage. It is possible observe that there
26 were significant statistical differences in the case of the flavonoids of the seedling roots for rutin, phorizine,
27 quercetin, galangin, and apigenin-7-0-glucoside. The highest concentrations of flavonoids: Phlorizin (737.1
28 µg/mL), quercetin (39.7 µg/mL), galangin (25.4 µg/mL), and apigenin-7-0-glucoside (9.95 µg/mL) were found
29 in the seedling roots whose seed was treated during 15 min. In the case of flavonoids rutin (3.4 µg/mL) was
30 found the maximum concentration in the radiation time of the seed for 5 min (Table 3-section a).

31
32 In relation to flavonoids of foliage it is possible observe that there were significant differences in phorizine,
33 quercetin, naringenin, galangin, hespiridin and apigenin-7-0-glucoside. Being, the highest concentration
34 obtained in the foliage of the seedlings of the control seeds (without radiation pre-sowing). For all cases, UV-
35 C radiation caused a decrease of this type of flavonoids in the foliage of the seedlings (Table 3-section b)

36
37

1

2

3 **Table 3** Concentration of flavonoids in bean seedlings from seeds radiated by UV-C: a) Roots, b) Foliage.

Flavonoids ($\mu\text{g/mL}$)	a) Root							Media
	T0 (Control)	T1	T2	T3	T4	DMS	R ²	
Rutin	1.9 ^a	0 ^b	3.4 ^a	0 ^b	1.9 ^a	1.5	0.958	1.45
Phlorizin	460 ^c	398 ^c	553.75 ^b	269.6 ^d	737.1 ^a	77.24	0.992	483.7400
quercetin	0 ^b	0 ^b	0 ^b	0 ^b	39.7 ^a	4.3294	0.997	7.936310
Naringenin	26.3 ^a	0 ^a	4.74 ^a	0 ^a	10.9 ^a	52.792	0.526	8.386
Phloretin	1.2594 ^a	0 ^a	0 ^a	1.6884 ^a	0 ^a	3.9597	0.525	0.589561
Galangin	17.32 ^b	7.52 ^d	15.85 ^b	11.5 ^c	25.48 ^a	4.608	0.982	15.53901
Naringin	185.8 ^a	48.2 ^a	41.57 ^a	39.82 ^a	26.64 ^a	190.1	0.756	68.41133
Hesperidin	198.7 ^a	185.69 ^a	139.24 ^a	117.15 ^a	195.21 ^a	214.8	0.430	167.2037
Apigenin-7-0- glucoside	0 ^c	5.4151 ^b	4.9966 ^b	3.57 ^b	9.95 ^a	0.0022	0.9857	4.788967
	b) Foliage							
Rutin	2.37 ^a	0.9368 ^a	0.1305 ^a	0 ^a	0.4258 ^a	2.7431	0.760633	0.773029
Phlorizin	532.9 ^a	515.1 ^a	247.2 ^c	411.2 ^b	268.4 ^c	62	0.991702	394.9824
Quercetin	162 ^a	54.46 ^b	5.36 ^b	0 ^b	0 ^b	101.6	0.93561	44.39249
Naringenin	3.53 ^a	1.02 ^b	1.89 ^b	13.0 ^a	0 ^b	10.506	0.865709	3.890745
Galangin	18.41 ^a	12.1 ^b	3.8 ^c	13.48 ^a	10.18 ^b	6.0233	0.952677	11.58393
Naringin	0 ^a	66.85 ^a	28.14 ^a	32.90 ^a	0.76 ^a	79.045	0.757594	25.73127
Hesperidin	249.32 ^a	82.75 ^b	0 ^b	1.67 ^b	11.27 ^b	163.66	0.915828	69.00097
Apigenin-7-0- glucoside	11.646 ^a	2.162 ^b	2.79 ^b	1.189 ^b	1.454 ^b	5.187	0.948951	3.859616

4 DMS is the least significant difference and the same letter are statistically equal (Tukey, $P \leq 0.05$). T₀, T₁, T₂,
5 T₃ and T₄ equals to 2, 5, 10 and 15 min of radiation UV-C.

6 In this way, this research demonstrates that by means of UV-C radiation, applied pre-sowing to the bean seed
7 (*Oti*), it is possible to modify phenolic compounds (phenolic acids and flavonoids) of the seedlings from the
8 radiated seeds. Similar results have been reported by Guajardo-Flores et al. (2014), who radiated the black bean
9 seed during 20 h in germination period, reporting higher accumulation of flavonoids during germination in
10 radiated seed. In this investigation, some of the flavonoids of the roots of the seedlings from radiated seed were
11 increased to 5 and 15 min of UV-C exposure.

12

13 As is known, secondary metabolites have a relevant role in the physiological and plant development processes,
14 included to stress responses and to the protection against pathogens (Katerova et al. 2013).

15 The results coincide with other authors who have detected the presence of flavonols, flavones and anthocyanins
16 in bean plants (Deckmyn et al. 1994). Especially the flavonoids are linked with regulatory mechanisms with
17 symbiosis of bacteria (M. A.W.B. and Phillips 1992). Coinciding the presence of naringin and naringenin in the
18 exudates of bean roots.

1 Other authors have agreed on the effects of UV-C radiation, but in another type of biological sample. As in the
2 case of callus culture; *e.g.* in callus culture (*Vitis vinifera* L.) where a higher accumulation secondary
3 metabolites was reported (Cetin 2014). Other authors applying the UV-C in callus culture of *Bacopa monnier*
4 have suggested that UV-C radiation is a useful technique which can be used to enhance the accumulation of
5 phytochemicals (Meenashree et al. 2018).

6
7 Likewise, the effects due to UV-C on secondary metabolites in epidermal tissues of fruit have been evaluated.
8 In tomatoes irradiated by UV-C, significantly increased total phenolic content was described when were
9 compared treated and untreated samples (Bravo et al. 2012).

10
11 According to our findings it has been demonstrated that the seed treated with UV-C light is degraded as a
12 function of the time of exposure to UV-C light, demonstrated through photoacoustic spectroscopy and scanning
13 electron microscopy. But, the seeds radiated could produce seedlings with higher concentration of some
14 secondary metabolites, as the flavonoid and phenolic acid type. These results could impact on different
15 applications, in the seed: to activate defense mechanisms and improve germination. Other future applications
16 could concentrate on the products consumed by the population, since they could modify the nutritional qualities
17 of the products consumed and have better nutritional quality of them.

18 For this reason, several recent studies have examined the importance of UV-C radiation with regard to different
19 quality parameters of treated plant foods, evaluating possible effects on their functional properties. A recognized
20 advantage of applying UV-C is the capability to improve the nutraceutical properties of plant foods, and some
21 studies have reported the possibility of increasing the antioxidant content through UV-C irradiation as a post-
22 harvest treatment. In this research, secondary metabolites were modified both in seed and in the seedling from
23 radiated seed. Then, in the case of agricultural seeds, UV-C radiation could serve not only to improve
24 physiological quality, but also physiological and nutritional, the latter being beneficial when the plant is
25 consumed in the form of sprouts.

26
27 In this way, UV-C radiation on seeds produce their degradation as a function of the radiation time. It is possible
28 that the UV-C light interacts with different structural levels of the seed and in this way to photostimulate
29 chromophores that intervene in metabolic processes of the seed. Future applications should be optimized
30 towards the most convenient method; in relation to the conditions of the seed, the characteristics of the seed
31 itself as well as the expected effect produced by UV-C light.

32 **CONCLUSIONS**

33 Variations of the photoacoustic signal produced by bean seeds exposed to UV-C radiation during 2, 5, 10 and
34 15 minutes, have been measured within the spectral band (270 - 400) nm. Based on scanning electron
35 microscopy, it has been shown that the decrease of this signal is generated by the seeds' degradation, such that
36 its maximum reduction from 0.24 to 0.10 mV shows up at 295 and 340 nm, after 15 minutes of exposure. As

1 the UV-C radiation time increases from 2 to 15 min, the morphological changes at the surface of the bean seeds
2 exhibits micro-holes and detachment of the cotyledon sclereids. The phenolic acids and flavonoids of the bean
3 seedlings of seeds treated by UV-C for different exposure times (0, 2, 5, 10 and 15 min) exhibit significant
4 statistical differences ($P \leq 0.05$) in both roots and foliage. For both the roots and foliage, the highest
5 concentration of phenolic acids has been found at 10 and 15 minutes, while for root flavonoids, it appears at 5
6 and 15 minutes.

7

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12 **Conflict of Interest**

13 There is no conflict of interest in relation to the article presented. Taking into consideration that the author and
14 the coauthors of the article mention that an original article is presented, not published and does not infringe any
15 legal copyright, or any property right or any other right whatsoever.

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