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Response of hydroponic baby lettuce to UV-B radiation exposure during the growing period

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Key words: Carotenoids, chlorophyll, dry matter, leaf area, polyphenols.

Abstract: Lettuce (*Lactuca sativa* L.) has a nutritional contribution comparable to other vegetables. It is produced in soil and hydroponics systems, outdoors or indoors, and in some cases, with the management of radiation. UV-B radiation exposure can influence the functional quality of vegetables and is becoming more frequent. Cultivars Kristine RZ and Versaï RZ were exposed to four radiation doses: UV-B0 (0 µW·cm^{-2}), UV-B16 (16 µW·cm^{-2}), UV-B33 (33 µW·cm^{-2}) and UV-B58 (58 µW·cm^{-2}), during 30 min for 10 days. Lettuce leaves were harvested twice. The leaf area of 'Versaï RZ' was not affected by radiation in the first harvest, while the high doses (33 and 58 µW·cm^{-2}) reduced the leaf area of 'Kristine RZ' between 15-30%, respectively. The radiation did not significantly impact the percentage of dry matter and the color parameters. However, functional compounds were affected. In general, the cv. Kristine RZ responded positively to the dose of 16 µW·cm^{-2} while 'Versaï RZ' to 58 µW·cm^{-2}. An increase in the content of functional compounds was also observed in 'Versaï RZ' in the second harvest, and a reduction in the levels measured in 'Kristine RZ' indicated a different adaptation to UV-B radiation that must be studied individually.

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All relevant data are within the paper and its Supporting Information files.

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1. Introduction

Lettuce (*Lactuca sativa* L.) is one of the most consumed leafy vegetables worldwide. Within the species, there are four botanical varieties with different characteristics: A) *L. sativa* var. *capitata*; B) *L. sativa* var. *longifolia*; C) *L. sativa* var. *crispa*; and D) *L. sativa* var. *acephala* (Kim *et al.*, 2016). It has a high water content (~95%), and despite its vast consumption, it is not considered an essential source of nutrient supply. However, its nutritional contribution is comparable to that of other vegetables because it is consumed raw, implying that the cooking processes do not affect its composition (Xiao *et al.*, 2012). Lettuce is low in calories, fat, and sodium and provides minerals, fibers, provitamin A or β-carotene, vitamins C, K, and folate (vitamin B9), and phenolic compounds to the diet, among others

(Kim *et al*., 2016).

The plasticity of the crop determines that it can be produced in soil and hydroponics systems. In addition, it can be harvested with different degrees of development ranging from the first leaves (cotyledons), seedlings (baby leaf), or fully developed plants (Xiao *et al*., 2012).

Hydroponics is a production system in which nutrients are supplied to plants artificially through water (Sharma *et al*., 2018). Three large hydroponic cultivation systems are differentiated into substrate, water, and air (aeroponics). The most used are water crops, which include two types: floating root system, where the crop is in continuous contact with the nutrient solution, and which have the advantages of being easy to perform, low cost, and do not require extra energy use; and the Nutrient Film Technique (NFT), which is a closed system where plants grown in a constant recirculation of a thin layer solution through the roots, with no loss or leakage of nutrient solution (Magwaza *et al*., 2020).

In addition to being the essential energy source for photosynthesis, light is one of the environmental factors that determine plants' growth, development, morphology, and synthesis of secondary metabolites. The relationship between light and plants has different conceptions and levels of complexity that involve aspects such as quality and quantity of light, which directly influence photosynthesis, but also the responses of plants to environmental stimuli. In this sense, many works have been developed on the ability of plants to detect and respond to the moment, duration, wavelength, dose, and direction of light, which involves the processes of photoperiodicity, phototropisms, and the photomorphogenesis (Robson *et al*., 2015).

The visible spectrum region, which goes from 400-700 nm, corresponds to the range of emissions they use and is known as photosynthetically active radiation (PAR). However, plants require a broader range for their development, which goes from 300 to 800 nm, which includes, in addition to PAR radiation, UV, and far red (Li and Kubota, 2009; Chory, 2010). One of the components of light is UV radiation, which according to its wavelength, is divided into UV-C (100-280 nm), UV-B (280-315 nm), and UV-A (315-400 nm). UV-C radiation and much of UV-B radiation (wavelengths less than 290 nm) do not reach the earth. UV-A radiation contributes approximately 5% of the photons in the photosynthetically active radiation (400-700 nm, PAR); it is highly variable, constituting no more than 0.33% of the photons in PAR. Although it represents a tiny fraction of the radiation that reaches the earth, it plays a fundamental role in regulating metabolic pathways in the development of the associated specific photomorphogenic responses (Robson *et al*., 2015; Robson *et al*., 2019). The study of the relationship of plants with UV-B radiation allowed the identification of a specific photoreceptor, the UV RESISTANCE LOCUS 8 (UVR8), which allowed a substantial advance in the understanding of signaling and response processes (Rai *et al*., 2021).

Exposure to UV-B radiation has a negative effect on photosynthesis due to damage at the level of DNA, proteins, and especially in the photosystems (PSI and PSII) and the light-harvesting complexes, which result from the increase in the levels of ROS. However, it is an effective elicitor to increase the content of bioactive compounds since one of the responses to exposure to UV-B radiation involves the induction and biosynthesis of phenolic compounds, including flavonoids, which act as UV protection components, and have antioxidant potential (Neugart and Schreiner, 2018). Different works mentioned increases in the concentrations of individual phenylpropanoids, such as hydroxycinnamates and flavonoids, in plants exposed to UV-B radiation. These changes are generally believed to positively impact the antioxidant capacity and UV protection (Moreira-Rodríguez *et al*., 2017 a, b; Dou *et al*., 2019; Rodríguez-Calzada *et al*., 2019; Castillejo *et al*., 2021; Loconsole and Santamaria, 2021). In the last 20 years, the consumption of vegetables has focused on the contribution of compounds of high nutritional value. Vitamins (E, C); hundreds of chemical compounds, such as sulfur and selenium; polyphenols such as flavonoids, stilbenes, and ellagic acid; and carotenoid compounds, such as lycopene, lutein, and ß-carotene among others are included in this group (Kyriacou *et al*., 2016). Consumers are looking for new products that promote health and longevity combined with gastronomic delight. Consequently, the way is opened to develop exceptional products that may be new, as in the case of microgreens or traditional products whose production systems have been modified (light management, for example) to influence their functional quality positively. Advances in the knowledge of physiological processes mediated by light have allowed the safe, healthy, and sustainable production of different plant species within controlled environments known as plant factories (SharathKumar *et al*., 2020; Yoon *et al*., 2022). Based on the above, the objective of this work was to evaluate the effect of the application of ultraviolet-B (UV-B) radiation under greenhouse conditions on the antioxidant characteristics of red and green "baby" lettuce leaves grown in a hydroponic system.

2. Materials and Methods

Plant material production

The study was carried out in a greenhouse at the Centro de Estudios de Postcosecha (CEPOC), at the Facultad de Ciencias Agronómicas de la Universidad de Chile (32°40' south latitude and 70°32' west longitude and 625 m a.s.l. altitudes, Santiago, Chile). Lettuce (*Lactuca sativa* L.), cultivars 'Kristine RZ' and 'Versaï RZ', both of oak leaf, green and red respectively, were used for the experiment. The sowing was carried out at the end of autumn in alveolate trays of 200 units, with a substrate of perlite with rock wool in a 1:1 ratio. At the first stages of the seedling, irrigation was made by tap water, depending on the requirements of the crop. Upon reaching the phenological stage of the first true leaf, the seedling was watered with a Hoagland II-modified nutrient solution, diluted to 50% with a pH between 5.5 and 5.8 measured with a potentiometer (Hi99301, Hanna Instruments, USA). Before sowing, a germination test was carried out according to ISTA Standards, obtained 96.7% for 'Kristine RZ' and 100% for 'Versaï RZ'. When the lettuces reached the stage of the third to fourth true leaf, they were transplanted to a 1.5x7 m NFT table with 8 profiles, a slope of 2.5%, and the height of the nutrient solution sheet of 0.005 m. Plant density was 53 plants m^{-2}. Once the transplant was carried out, the crop was irrigated continuously with tap water for 5 days to reduce the stress of the transplant. When plants were in the phenological stage of the four to fifth true leaf, they were irrigated with a Hoagland II-modified nutrient solution diluted to 50%. During cultivation, pH conditions were between 5.5 and 5.8, measured with a potentiometer (Hi99301, Hanna Instruments, USA).

UV-B irradiation treatments

Before treatments applying, photosynthetically active radiation (PAR, µmol m^{-2}s^{-1}) was measured with a PAR radiation meter (Fieldscout, Model 3415, Spectrum Technologies, Inc., Illinois, USA) to determine the best time for UV-B irradiation. The chosen time was at 7:00 p.m., which corresponded to the moment closest to the point of light compensation of sun plants (20-30 µmol m^{-2} s^{-1}); where the photon flux in the net exchange of the leaf is zero, equalizing the rates of production and consumption of CO_2 (Yin *et al*., 2011).

UV-B radiation was applied with 13 UV radiation lamps (Q-Panel 313, Cleveland, USA), arranged in a steel structure of 1.5x1.8 m. The lamps were covered with a 0.11 mm mica (Socomish, Santiago, Chile) to isolate any other type of radiation other than UV-B radiation. Four radiation doses corresponding to: UV-B0 (0 µW·cm^{-2}), UV-B16 (16 µW·cm^{-2}), UV-B33 (33 µW·cm^{-2}) and UV-B58 (58 µW·cm^{-2}) were evaluated. Radiation was applied for 30 min daily for 10 days when the lettuces reached the fifth to sixth true leaf. Greenhouse growth conditions were 22.4±4.5°C mean daily temperature and 48.8±6.5% mean daily relative humidity.

After 10 days, when the lettuces were in the phenological stage of 8th to 9th true leaf, 7 to 8 outer leaves were harvested, leaving 2 to 3 leaves per plant. These plants remained in growing conditions until they reached 5 to 6 fully extended leaves the moment they were irradiated. When plants reached the same condition as in the first harvest, they were harvested again (second harvest). The time difference between the first and second harvests was 19 days.

At each moment of analysis, the following determinations were made:

Leaf area (cm^2). Measured in a total of 30 leaves per variety, with a leaf area determiner (Area Meter, LI-COR 3000, USA).

Fresh and dry matter of the aerial part (g). Weight of whole leaves was determined with a precision balance (Radwag, AS 100/C/2, Poland) and corresponded to the fresh matter. After that, leaves were dried in an oven (Labtech, LDO-150F, Korea) with forced air ventilation at 70°C until constant mass to obtain the dry mass. Values were expressed in percentages.

Colour. Measured in the adaxial part of the distal sector of the lamina in 30 leaves per variety, using a compact tristimulus colorimeter (Minolta Chroma meter, CR-300, Ramsey, NJ, USA) with a D65 light source, an angle observed from 0° and calibrated with a white standard, using the CIELab system. Parameter values were expressed as hue (h_{ab}), chroma (C*), and lightness (L*).

Chlorophyll a, b, and carotenoids determination *(mg g^{-1}).* It was carried out according to the methodology proposed by Lichtenthaler and Wellburn (1983). For the extraction, 0.4 g of the distal part of the leaf blades were weighed (Radwag, AS 100/C/2, Poland), and 15 mL of 80% (v/v) acetone were added. Subsequently, the mixture was homogenized at 3,500 rpm for 30 s (IKA T18 basic, Ultra Turrax, Wilmington, USA), filtered with gauze, and centrifuged (HERMLE Labortechnik, Z326K, Wehingen, Germany) for 15 min at 3,630 gx. The determinations were made in the supernatant that was measured in a plate spectrophotometer (Asys, UVM340, Eugendorf, Austria) at 470, 646, and 663 nm. For the quantification, the following expressions were used:

$$Ca = 12.25\ A_{663} - 2.79\ A_{646}$$

$$Cb = 21.5\ A_{646} - 5.1\ A_{663}$$

$$C_{x+c} = \frac{1000\ A_{470} - 1.82\ Ca - 85.02\ Cb}{198}$$

Where C*a* is the chlorophyll *a* content, C*b* the chlorophyll b content and C_{x+c}, the carotenoid content.

Extraction of bioactive compounds. It was made following the methodology proposed by Swain and Hillis (1959) with some modifications. For this, 5 g of sample were weighed (Radwag, AS 100/C/2, Poland), mixed with 20 mL of methanol, and homogenized at 3,500 rpm for 45 s (IKA T18 basic, Ultra Turrax, Wilmington, USA). The homogenate was stored at 5°C for 24 h. Subsequently, it was filtered with gauze and centrifuged (HERMLE Labortechnik, Z326K, Wehingen, Germany) for 20 min at 3,630 x *g*. Measurements were made on the supernatant.

Total phenolic compounds (µg GAE g^{-1}). It was determined according to the colorimetric method of Folin Ciocalteu (Singleton and Rossi, 1965), placing 19.2 µL of extract/blank, together with 29 µL of Folin-Cioclateu reagent (1:8 v/v with distilled water) in each cell of the Elisa plate. After 3 min, 192 µL of 1N Na_2CO_3 were added, and 10 min after, the time at which the reaction was complete as previously determined; absorbance was measured at 750 nm. For the calculation, a calibration line was made with gallic acid (R^2= 0.9958). The values were expressed in µg of gallic acid equivalent (GAE) g^{-1} of fresh weight.

Antioxidant capacity by DPPH (µg ET·g^{-1}). It was determined according to the methodology of Brand-Williams *et al.* (1995), placing 21 µL of sample and 194 µL of DPPH solution in each cell (previously adjusted to 1.1 absorbance at 515 nm). After 2 h, at which time the reaction was complete, the absorbance was measured. For the calculation, a calibration curve was made with Trolox (R^2= 0.9992). The results were expressed as µg Trolox equivalent (TE) g^{-1} fresh weight.

Antioxidant capacity by FRAP (µg ET·g^{-1}). For the analysis, the methodology proposed by Benzie and Strain (1996) was followed. To 6 µL of sample, 198 µL of FRAP reagent was added (buffer acetate 300 mM pH 3.5 + ferric chloride 20 mM aqueous solution + 2,4,6-Tripyridyl-s-Triazine 10 mM in HCl 40 mM). After 30 min, time in which the reaction stabilized, the absorbance at 593 nm was measured. A calibration curve was made with Trolox (R^2= 0.9951) to express the results as µg Trolox (ET)·g^{-1} fresh weight.

Statistical analysis

The experimental design was a 4x2 factorial, completely randomized with 3 repetitions. The factors corresponded to the level of UV-B radiation and cultivar, being distributed randomly within each repetition. The experimental unit used was 8 plants per replicate and cultivar.

An analysis of variance (ANDEVA) was performed, and when statistically significant differences were found, Tukey's multiple range comparison test was used, with a significance level of 5%.

The percentage values were corrected prior to statistical analysis using the following formula:

$$Corrected\ value = arcsen\sqrt{y/100}$$

where *y* is the percentage values (0 to 100).

3. Results

The foliar leaf area showed differences between genetic materials and treatments in the first harvest (Fig. 1A). The foliar leaf of cv. 'Kristine RZ' was more significant than cv. 'Versaï RZ on control and lower UV-B radiation (16 µW.cm^{-2}). When supplemented with UV-B radiation, no response was found in the cv. Versaï RZ. However, the higher doses of radiation (33 and 58 µW·cm^{-2}) reduced the leaf area of 'Kristine RZ'. Nevertheless, UV-B radiation doses applied to each cv. in the second harvest did not differ. Differences were only observed between 'Kristine RZ' and 'Versaï RZ', being the leaf area higher in the first one (Fig. 1B).

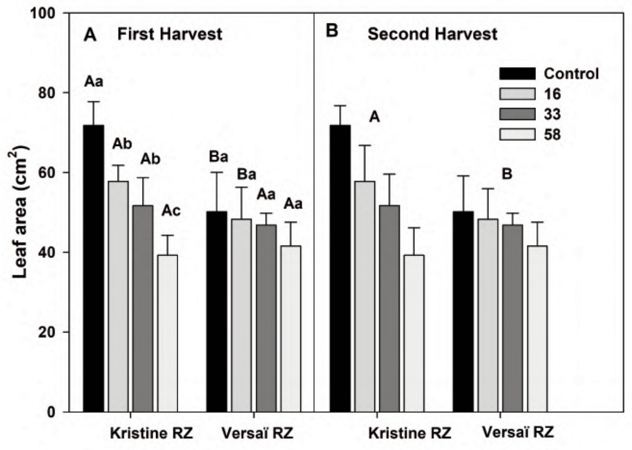

Fig. 1 - Effect of UV-B radiation on leaf area (cm²) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 10). Different letters, uppercase for cultivars and lowercase for treatments, indicate significant differences according to Tukey's test (p≤0.05).

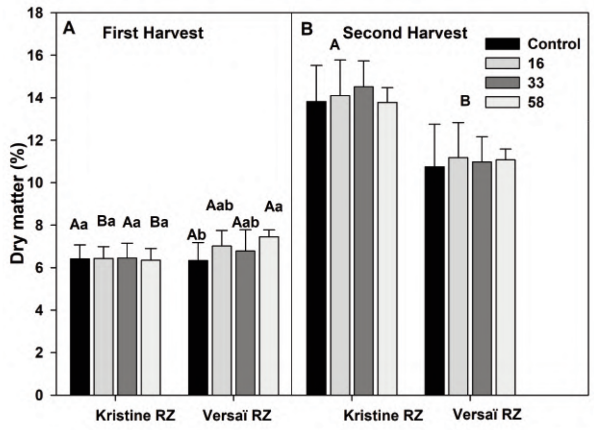

Fig. 2 - Effect of UV-B radiation on dry matter (%) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 10). Different letters, uppercase for cultivars and lowercase for treatments, indicate significant differences according to Tukey's test (p≤0.05).

The Kristine RZ cultivar dry matter did not show significant differences between the intensities of UV-B radiation in the first harvest, with an average of 6.4% (Fig 2A). In ‘Versaï RZ,’ differences were only observed between the control and 58 µW·cm^{-2}. The cultivar effect was expressed in a higher dry matter/fresh matter ratio by the cultivar ‘Versaï RZ’ compared to ‘Kristine RZ’ in the UV-B radiation intensities of 16 and 58 µW·cm^{-2}; while at 0 and 33 µW·cm^{-2}, there were no significant differences between cultivars. In the second harvest, the cv. Kristine RZ presented a higher percentage of dry material (~13%) compared to ‘Versaï RZ’ (~11%), without differences related to the intensity of the radiation being observed in any of them (Fig. 2B).

UV-B radiation did not significantly affect the different color parameters of the evaluated lettuce (Table 1). The ‘Kristine RZ’ presented h_{ab} values of 120° corresponding to green coloration, while ‘Versaï RZ’ presented values of 90° indicating red-yellowish coloration. The cv. Kristine RZ did not show variations in this parameter either in the first or the second harvest. In the case of ‘Versaï RZ,’ the behavior was

Table 1 - Lettuce cultivars used in this experiment

Treatment	First harvest		Second harvest	
	'Kristine RZ'	'Versaï RZ,'	'Kristine RZ'	'Versaï RZ,'
*L**				
Control	121.81 ± 1.17 Aa	72.14 ± 0.88 Ba	117.85 ± 1.57 Aa	30.92 ± 1.78 Bc
16	121.89 ± 1.38 Aa	67.03 ± 1.13 Bb	117.41 ± 1.14 Aa	25.96 ± 0.67 Bd
33	122.53 ± 1.06 Aa	72.41 ± 0.88 Ba	117.81 ± 1.27 Aa	33.42 ± 0.23 Bb
58	121.47 ± 1.04 Aa	67.52 ± 0.96 Bb	117.69 ± 1.65 Aa	37.66 ± 0.18 Ba
hab				
Control	41.25 ± 0.57 Aa	8.45 ± 1.11 Ba	45.94 ± 1.63 Aa	7.33 ± 0.95 Bb
16	40.12 ± 0.72 Aab	8.33 ± 0.88 Ba	46.73 ± 0.16 Aa	7.99 ± 1.16 Bab
33	38.41 ± 2.69 Abc	7.95 ± 0.66 Ba	45.44 ± 2.38 Aab	8.58 ± 1.21 Bab
58	37.72 ± 1.23 Ac	8.09 ± 0.85 Ba	44.14 ±0.28 Ab	9.06 ± 0.28 Ba
*C**				
Control	60.79 ± 0.53 Aa	34.32 ± 0.47 Ba	64.78 ± 2.16 A ns	32.01 ± 3.74 B ns
16	59.77 ± 0.81 Aab	33.74 ± 0.72 Bab	64.02 ± 2.51 A	32.42 ± 2.27 B
33	57.31 ± 1.55 Ab	33.66 ± 1.63 Bb	62.81 ± 1.27 A	32.31 ± 3.27 B
58	57.16 ± 1.38 Ab	33.11 ± 0.16 Bb	64.14 ± 1.72 A	32.72 ± 2.69 B

Each value was indicated by mean±standard error (n=8). Different letters indicate significant differences by Tukey's multiple test with a significance level of 0.05.

quite erratic since, in the first harvest, the leaves of the control and the 33 μW·cm^{-2} treatment were the least red. Compared to the first harvest, in the second, the leaves of 'Versaï RZ' were redder, with values between 25-35, especially those of the control and the 16 μW·cm^{-2} treatment.

Regarding saturation expressed by C*, 'Kristine RZ' presented higher values than 'Versaï RZ' in both harvests, indicating more vivid colors. It also presented greater luminosity (L*). Higher UV-B radiation intensities in the first harvest in 'Kristine RZ' generally reduced saturation and lightness (C* and L* respectively). While in 'Versaï RZ,' only a decrease in luminosity was observed. In the second harvest, the behavior of 'Kristine RZ' was like that of the first. However, in 'Versaï RZ,' the effect was the opposite since the greater intensity of radiation determined a greater saturation. However, the parameter L* was not affected by radiation in either of the two cultivars.

Chlorophyll *a* content showed differences between genetic materials and radiation levels. In the first harvest, 'Kristine RZ' presented about 10 times more than 'Versaï RZ' in all treatments. In 'Kristine RZ,' the highest values were measured in control and 16 μW.cm^{-2} , while the lowest was in those with higher radiation intensities, with no differences between them. On the contrary, the treatments did not affect chlorophyll's *a* level of 'Versaï RZ.' (Fig. 3A).

In the second harvest, the behavior was practically the opposite. In most treatments, 'Kristine RZ' presented lower levels of chlorophyll than cv. Versaï RZ. No response to treatments was found in 'Kristine RZ,' while in 'Versaï RZ,' there was an increase in treatments of 33 and 58 μW·cm^{-2}, respectively (Fig. 3B). Comparing the values measured in the first and second harvest, in the cv. Kristine RZ, the values were practically halved. At the same time, in 'Versaï RZ,' it increased between 3 and 9 times, indicating a very different response to radiation linked not only to the genotype but also to the age of the plant.

Chlorophyll *b* values measured at the first harvest in cv. Kristine RZ were around 10 higher than those of 'Versaï RZ.' In both, an effect of UV-B radiation levels was observed. In 'Kristine RZ,' the 16 μW·cm^{-2} treatment determined an increase, while in 'Versaï RZ,' the increase was observed in the 58 μW·cm^{-2} treatments (Fig. 4A). In the second harvest, only differences between varieties were observed (Fig. 4B). However, contrary to the first, the values measured in 'Kristine RZ' were around half of those measured in 'Versaï RZ.'

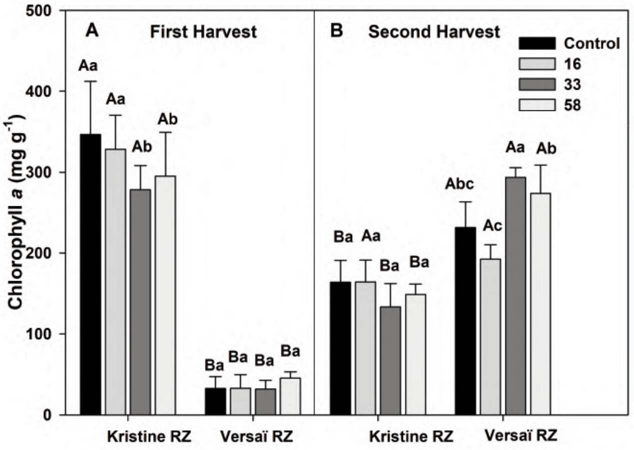

Fig. 3 - Effect of UV-B radiation on chlorophyll a content (mg·g^{-1}) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 3). Different letters, uppercase for cultivars and lowercase for treatments, indicate significant differences according to Tukey's test (p≤0.05).

Also, in the case of carotenoids, both in the first and in the second harvest, differences were found between genetic materials. While, in the first harvest, 'Kristine RZ' presented around 10 times more, in the second, it presented between 37-62% less (Fig. 5A and B). Regarding the effect of the intensity of the radiation, in the first harvest the two highest doses of UV-B radiation had a negative effect on the carotenoids of 'Kristine RZ', while the intensity did not affect the carotenoid levels of 'Versaï RZ'. On the

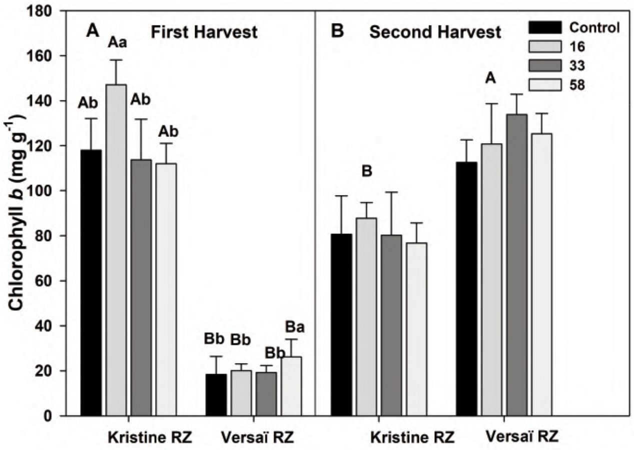

Fig. 4 - Effect of UV-B radiation on chlorophyll b content (mg·g^{-1}) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 3). Different letters, uppercase for cultivars and lowercase for treatments, indicate significant differences according to Tukey's test (p≤0.05).

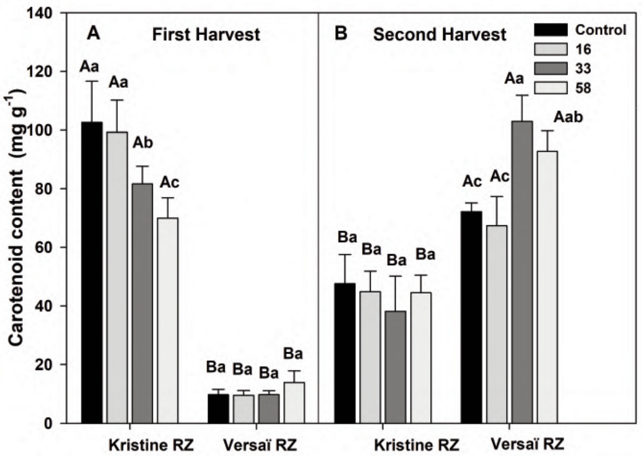

Fig. 5 - Effect of UV-B radiation on carotenoid contend (mg·g^{-1}) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 3). Different letters, uppercase for cultivars and lowercase for treatments, indicate significant differences according to Tukey's test (p≤0.05).

contrary, in the second harvest the radiation did not cause differences in the content measured in 'Kristine RZ' but determined in 'Versaï RZ' lettuces exposed to 33 and 58 µW·cm^{-2} an increased.

At both harvest times, 'Versaï RZ' presented about 70-80% more total phenolic compounds than 'Kristine RZ.' In 'Versaï RZ' from the first harvest, the radiation intensity did not affect the values (Fig. 6A). In the second harvest, an increase in phenolic compounds was observed in both cultivars, independent of the radiation dose (Fig. 6B).

Regarding CAT measured by the DPPH method, both in the first and second harvest, 'Versaï RZ' surpassed 'Kristine RZ,' which presented between 3 and 5 times fewer antioxidant compounds (Fig. 7). In the first harvest, there was no response to radiation in the case of 'Kristine RZ,' while in 'Versaï RZ' the intensities of 16 and 33 µW.cm^{-2} reduced the levels of these compounds (Fig. 7A). However, in the treatment of 58 µW.cm ^{2}, there were no differences with the control. In the second harvest, the response to the dose was somewhat erratic in 'Kristine RZ'. At the same time, in 'Versaï RZ', the control treatment presented the lowest levels while the radiation determined an increase, reaching the highest values at 16 µW·cm^{-2} (Fig. 7B).

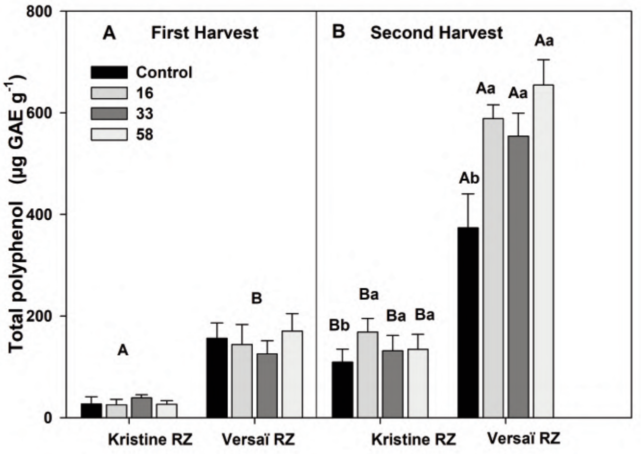

Fig. 6 - Effect of UV-B radiation on total polyphenol content (µg GAE. g^{-1}) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 3). Different letters, uppercase for cultivars and lowercase for treatment, indicate significant differences according to Tukey's test (p≤0.05).

On the other hand, when CAT was measured by the FRAP method, both in the first and in the second harvest, differences were observed between genetic materials, with 'Versaï RZ' being superior to 'Kristine RZ' (Fig. 8).

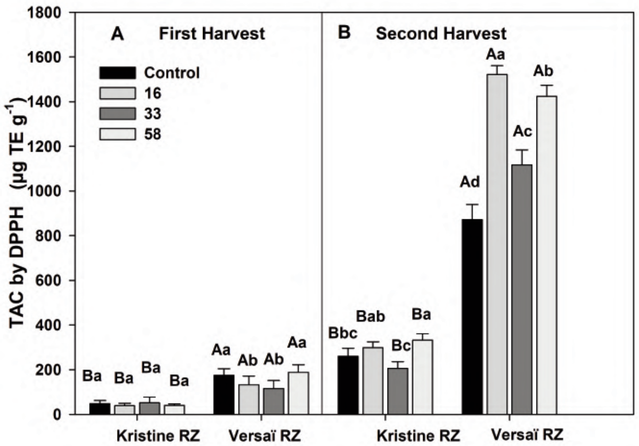

Fig. 7 - Effect of UV-B radiation on total antioxidant capacity by DPPH method (µg TE·g^{-1}) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 3). Different letters, uppercase for cultivars and lowercase for treatments, indicate significant differences according to Tukey's test (p≤0.05).

4. Discussion and Conclusions

There are reports that the application of supplemental UV-B radiation has a negative effect on vegetative growth in general and consequently on the yield of different plant products. The most frequently reported alterations include a decrease in the leaf area and/or an increase in the thickness of the leaves. Plant exposure to UV-B radiation, both in the field and in controlled environments, reduced leaf development in lettuce, peas, corn, and sweet pepper, among others (Choudhary and Agrowal, 2014;

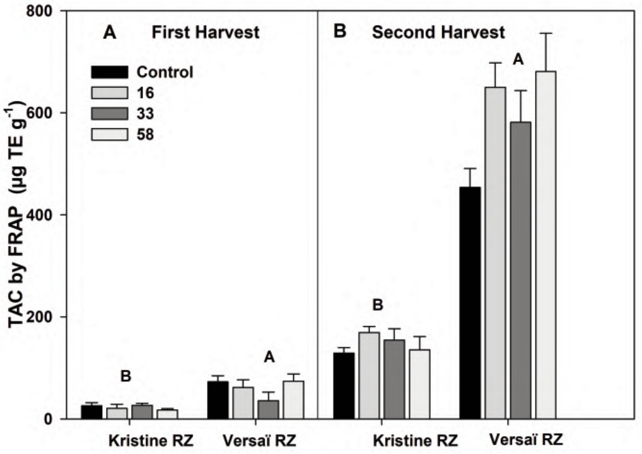

Fig. 8 - Effect of UV-B radiation on total antioxidant capacity by FRAP method (µg TE·g^{-1}) of baby lettuce 'Kristine RZ' and 'Versaï RZ' in a hydroponic system, on first (A) and second harvest (B). Values are means (n= 3). Different letters, uppercase for cultivars and lowercase for treatments, indicate significant differences according to Tukey's test (p≤0.05).

Fina *et al*., 2017; Rodríguez-Cazalda *et al*., 2019). Although alterations occur because of exposure to UV-B radiation, it is mentioned that they could be transitory since, once the acclimatization stage has been overcome, which involves processes such as the positive regulation of ROS elimination, the detection UV and DNA repair capabilities; any interruption in leaf development is overcome. This may allow leaf development to resume its original pattern, or even produce a compensatory response whereby greater expansion is matched by reduced division (Héctors *et al*., 2010; Robson *et al*., 2015). This could be explaining the behavior observed in the second harvest.

On the other hand, and contrary to what was found in this work, it is indicated that exposure to UV-B radiation reduces plants' biomass due to the lower capacity for photosynthesis, mainly due to the effect that UV-B radiation could have. B on photosystem II (PSII) (Bornman, 1989; Mittal *et al*., 2021). Another factor involved is the reduction in the amount of chlorophylls that negatively affects biomass (Kataria and Guruprasad, 2012) as well as the lower turgor pressure that prevents cells from increasing their water content (Choudhary and Agrawal, 2014; Fina *et al*., 2017). More recently, Rizi *et al*. (2021) pointed out that exposure to UV-B rays negatively affects many compounds and biochemical processes in plants, including chlorophyll content and photosynthesis, which reduces carbohydrate production, with the consequent adverse effect on growth and biomass.

The changes observed in color are related to those observed in the different compounds, both pigments, chlorophyll a, b, and carotenoids, as well as phenolic compounds, which include others also linked to color, such as anthocyanins (Goto *et al*., 2016; Sytar *et al*., 2018; Gurdon *et al*., 2019). The differential response found in the varieties studied was also observed by other authors. UV-B radiation induces physiological, biochemical, and morphological stress responses in plants, which are species-specific and even differ between cultivars. In a study where two blueberry cultivars (Legacy and Bluegold) were analyzed, a different response was found where in Legacy (resistant to UV-B radiation) there was an increase in photoprotective pigments during the first week of exposure (19 µW·cm^{-2}) and from the second there was a reprogramming of its metabolism that determined an increase in phenolic compounds and its antioxidant capacity (Luego Escobar *et al*., 2017).

The differences found between the first and second harvests may be linked to the lettuce varieties presenting differential acclimatization mechanisms. In this sense, 'Kristine RZ' has an immediate response, but tolerates low levels of radiation. On the other hand, 'Versaï RZ' takes longer to adapt to UV-B radiation. However, it is capable of responding to higher radiation doses. The differential response is because after exposure to radiation, plants need to reprogram their metabolism to alleviate stress (Barnes *et al*., 2015; Wargent *et al*., 2015). On the other hand, the answer will depend on the type of pigment being considered, which in the case of carotenoids comprises different molecules with different sensitivity to UV-B radiation (Badmus *et al*., 2022).

In a study carried out on broccoli sprouts, the application of 0.042 W·m^{-2} for 4h + 24h of adaptation did not determine variations in carotenoids or chlorophylls (Mewis *et al*., 2012). However, when broccoli sprouts were treated with 7.16 W·m^{-2} for 120 min, photoreceptor pigments were differentially affected, determining increases in carotenoids, lutein and mainly neoxanthin, and in chlorophyll *a*, in relation to control (Moreira-Rodríguez *et al*., 2017 a). According to León-Chan *et al*. (2017), the daily exposure of pepper, during growth (days), to 72 kJ·m^{-2} for 6 h, did not alter the levels of chlorophyll *a* and *b* in relation to the control. However, it determined a notable increase in carotenoids (from 0.02 to 2.18 mg·100 g^{-1} FW).

To protect themselves from the damage generated by UV-B radiation, plants activate their defense mechanisms to avoid excess ROS and maintain the stability of their cellular structures. The biosynthesis of antioxidant compounds, among which are those of a phenolic nature (phenolic acids, flavonoids, among others) as well as vitamins, is one of the defense mechanisms (León-Chan *et al*., 2017; Moreira-Rodríguez *et al*., 2017b; Neugart and Schreiner, 2018). In a study carried out with basil exposed to different doses of UV-B radiation (8.5, 34, 68, 102 kJ m^{-2} day^{-1}), it was found that discontinuous applications for long periods (about 6 days) determined an increase in phenolic compounds without altering the photosynthetic process, directly proportional to the dose of radiation used (Mosadegh *et al*., 2018). In a similar work with purple and green basil exposed to 18.7 kJ m^{-2} h^{-1} for different exposure times, increases in the concentrations of anthocyanin, phenols, and flavonoids were found that even reached 169% (Dou *et al*., 2019). Therefore, it is expected that exposure to UV-B radiation will increase, as observed in the lettuce varieties studied. Castillejo *et al*. (2021) applied doses of 5, 10, and 15 kJ·m^{-2} to kale sprouts during germination at 3.5, 7, and 10 days (25% of the dose at each moment) and found variations in the levels of antioxidant compounds. Doses of 10 and 15 kJ .m^{-2} increased phenol levels by 30%. In addition, TAC experienced increases of 10% (measured by DPPH) and 20% (measured by FRAP) because of the protection mechanism of plants against the stress factor constituted by UV-B radiation.

In work carried out by Hao *et al*. (2022) in Pak Choi, an increase in the amount of phenolic compounds measured by DPPH and FRAP was found, depending on the applied radiation dose. Doses of 0.7 W.m^{-2} for 4 and 8h determined increases. However, no response was observed when the radiation increased to 1.4 W·m^{-2} or the exposure time was greater than 8 h. The authors attribute this to the fact that different signaling pathways are activated depending on the dose.

A response linked to genetic characteristics and plant age was also observed, but it did not follow the same pattern as for chlorophylls and carotenoids. In this case, both varieties took longer to acclimatize, so the most critical response corresponded to the second harvest. In this sense, Rizi *et al*. (2021) reported an increase in both phenolic compounds (1.34 times) and flavonoids (2 times) concerning the control and after 5 days of exposure to radiation of 10.97 kJ m^{-2} day^{-1}, especially in the young leaves of *salvia verticillata*.

Therefore, controlled doses of UV-B radiation can be used to develop products with added value as they are rich in functional compounds, as shown in this and other works. These applications must be evaluated in each genetic material to adjust the dose, as well as the behavior of the plants since it has been demonstrated once again that the response is specific.

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