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Applied botany: UV-B radiation to obtain quinoa sprouts enriched in phenolics, with probable uses in natural sunscreen protectors or as functional foods

Botánica aplicada: radiación UV-B para la obtención de brotes de quinua enriquecidos en compuestos fenólicos, con probables usos en protectores solares naturales o como alimentos funcionales

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ABSTRACT

The objective was to evaluate the use of a microgreen system and very short UV-B radiation dose to obtain plant biomass as a source for phenol-enriched extracts with probable cosmetic and/or food uses. Quinoa seedlings, a native species of the Andes, of two different ages were used. The seedlings were irradiated with different doses of UV-B and then evaluated by quantifying indicators of oxidative damage. Also, the contents of phenolic compounds, photosynthetic pigments, antioxidant capacity, and sun protection factor were determined. The results showed that the youngest seedlings responded better to short UVB doses, increasing the content of soluble

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and insoluble phenols, without showing oxidative damage. These results were correlated with the greater antioxidant power of the extracts and an intermediate sun protection factor. We conclude that this species, grown in a microgreen system, is a promising alternative to obtain phenol-enriched extracts with possible use in formulations of natural sunscreens. In this sense, these results can serve as a starting point for optimization studies through the response surface methodology.

Keywords: Microgreen; phenolics; quinoa; sunscreen; UB-V.

RESUMEN

El objetivo fue evaluar el uso de un sistema de microgreen y dosis muy cortas de radiación UV-B para la obtención de biomasa vegetal como fuente de extractos enriquecidos en compuestos fenólicos con probables usos cosméticos y/o alimentarios. Se utilizaron plántulas de quinua, especie nativa de los Andes, de dos edades diferentes. Las plántulas fueron irradiadas con diferentes dosis de UV-B y luego evaluadas cuantificando indicadores de daño oxidativo. Además, se determinó contenido de compuestos fenólicos, pigmentos fotosintéticos, capacidad antioxidante y factor de protección solar. Los resultados mostraron que las plántulas más jóvenes respondieron mejor a dosis cortas de UVB, aumentando el contenido de fenoles solubles e insolubles, sin presentar daño oxidativo. Estos resultados se correlacionaron con el mayor poder antioxidante de los extractos y un factor de protección solar intermedio. Concluimos que esta especie, cultivada en un sistema de microgreen, es una alternativa prometedora para la obtención de extractos enriquecidos en compuestos fenólicos con posible uso en formulaciones de protectores solares naturales. En este sentido, estos resultados pueden servir como punto de partida para estudios de optimización mediante la metodología de superficie-respuesta.

Palabras clave: Fenólicos; microcultivo; pantalla solar; quinoa; UV-B.

INTRODUCTION

Ultraviolet radiation (UVR) reaching the earth is largely composed of UV-A radiation and to a lesser extent UV-B. Although UVR is known to vary greatly at the Earth's surface for various reasons (Kerr, 2005), in the last decades, the ozone layer has been substantially damaged because of several anthropogenic activities, which increased the UVR reaching the Earth. UV-B radiation is considered 1000 times more dangerous than UV-A radiation because it is genotoxic and causes skin burns (Brenner and Hearing, 2008; Lorigo *et al*., 2018). Overexposure to UVR increases the production of free radicals that produce alterations in macromolecules such as lipids,

proteins, and nucleic acid or induce activation of responsible enzymes of cleavage of extracellular matrix components (Orqueda *et al*., 2021). In this sense, topical sun protective cosmetics, both sunscreens, pre- and post-sun, have been intensively developed and produced to protect human skin against damages or pathologies associated with solar irradiation. Most sun protection cosmetics contain synthetic chemical substances, such as titanium dioxide nanoparticles, whose use has been questioned since adverse effects on cellular oxidative stress (Falck *et al.*, 2009; Krishnaiah *et al.*, 2009; Grande and Tucci, 2016; Ferraro *et al*., 2020). In addition, many of the components of sunscreens have been detected in natural water sources and are currently considered emerging contaminants (Kim *et al*., 2011; Simonin *et al*., 2016; Prakash and Anbumani, 2021).

In this context, plant-derived bioproducts have gained considerable attention as skin-protecting agents. Currently, some studies have focused on the use of plant extracts rich in secondary metabolites, such as polyphenols and flavonoids, which show antioxidant activity. Thus, they can act on free radicals generated by exposure to UV-B radiation, reducing the probability of the occurrence of cancer or cell aging (Birt *et al*., 1997; Takshak y Agrawal, 2019; Lima-Cherubim *et al*., 2020; Pavelkova *et al*., 2020). In addition, phenolic compounds have also been attributed anti-inflammatory and antimicrobial functions in several biological systems making them excellent candidates to be added to cosmetic formulations (Ribeiro *et al*., 2015; Lima-Cherubim *et al*, 2020; Salas *et al*., 2020; Orqueda *et al*., 2021; Orqueda *et al*.,2022).

During evolution, plants have developed the ability to produce an enormous number of secondary metabolites, which are essential for their interaction with the environment, their reproductive strategies, and defense mechanisms (Cheynier *et al*., 2013; Li *et al*., 2018). For this reason, native plants from adverse environments have high contents of secondary metabolites, which help them survive in such environments. Quinoa (*Chenopodium quinoa*) is a species native to the Andean region of Bolivia, Ecuador, Chile, and Argentina. Due to its location, it is adapted to grow up to 3500 m above sea level, so it has developed different strategies to tolerate various adverse conditions, such as high levels of UV-B radiation, low temperatures, drought, etc. Among these strategies, it can mention the synthesis of phenolic compounds that act like screens and antioxidant molecules. In this sense, Hilal *et al.* (2004) studied the effect of UV-B radiation in quinoa seedlings, and they demonstrated an increase of absorbent phenolic compounds in the cotyledon epidermis. Also, other studies have shown that the synthesis of phenolic compounds varies with the dose of UV-B received and with the age of the organ studied (Huarancca Reyes *et al.*, 2018; Wittayathanarattana *et al.*, 2022). The studies carried out by these authors include plants over one month old subjected to prolonged treatments (4 to 16 hours) with UVB and white LED lamps, simultaneously. In this sense, to achieve efficient production, the use of microgreens could be

a profitable option to obtain sufficient biomass to produce plant extracts enriched in phenolic compounds through controlled stimulation of protective mechanisms against stress conditions. Likewise, UVB-stimulated quinoa sprouts could serve as a functional food option, with higher content of phenolic compounds (Al-Qabba *et al*., 2020; Ng and Wang, 2021). The objective was to evaluate the use of a microgreen system and very short doses of UV-B radiation to obtain plant biomass as a source of extracts enriched with phenolics or as a food alternative.

MATERIALS AND METHODS

Plant material and light treatments

Quinoa seeds (*Chenopodium quinoa*) were provided by INTA of San Juan, Argentina. Seeds were germinated and grown in plastic boxes (30 cm x 15 cm x 3 cm) containing vermiculite moistened with tap water. The germination and growth plants were carried out in a growth chamber with controlled conditions of temperature (25 ±1°C), and a photoperiod of 12 h (PAR 400–700 nm of 180 μmol/m/sec).

The UV-B radiation was supplied by two fluorescent lamps (UB-B-313, Q-Panel, Cleveland, OH) mounted horizontally 15 cm above the tops of the plants. Seven and ten days-old seedlings were irradiated with UV-B (4,12 W/m^2) to three doses: for 3, 10 or 30 minutes at the middle of the photoperiod (in the absence of PAR radiation). Seven and ten days correspond to the stage of greatest activity of the cotyledons prior to the beginning of senescence (Ruffino *et al.*, 2008).

The irradiance at the plant level was determined with a radiometer (Series 9811, Cole-Parmer Instrument Co., Chicago, IL). To exclude UV-B radiation for control plants treated, the radiation was filtered through cellulose acetate. Following irradiation, the seedlings were returned to initial growth conditions for 24 h. At 8 or 11 days, the samples of cotyledons were collected. The plant tissues selected did not show signs of chlorosis and/or morphological alterations and they were kept at -4° C (tissue fresh) until chemical determinations.

Extraction and determination of soluble phenolics (SP) and insoluble phenolics (IP)

Soluble phenolics (SP) were extracted according to Swain and Hillis (1959) with minor modifications. Briefly, samples of fresh cotyledons and true leaves (250 mg FW) were homogenized with 3 mL 96 % ethanol, incubated for 48 h at room temperature and darkness, and finally centrifuged at 3000 g for 5 min. Supernatants were recovered and used for SP determination. Aliquots of supernatants (50 μl) were added to 0.2 mL (1:1 v/v) of Folin–

Ciocalteu reagent and 1.95 mL of distilled water, then it was left to rest at room temperature for 2 min. Finally, 0.8 mL of 7.5 % Na_2CO_3 was added and incubated for 5 min at room temperature. The absorbance was read at 760 nm. Precipitate from SP extraction was washed twice with 2 mL ethanol 96% and centrifugation at 3000 g for 5 min. Washed precipitate was dried at 37 °C for 48 h and used to obtain IP (cell wall-bound phenolics). IP extraction was adapted from Assabgui *et al*. (1993). Dried samples (20 mg) were hydrolyzed with 2 mL of 2 N NaOH in a water bath at 60 °C for 60 min. The solutions were cooling and slowly acidified up to pH 2.0 with 5 N HCl and extracted with ethyl acetate. After, ethyl acetate fractions were taken near dryness under a stream of N_2 gas and dissolved in 0.5 mL of 96 % ethanol. Insoluble phenolics were determined using the Folin–Ciocalteu reagent as described above. SP and IP concentrations were determined using a standard curve made with gallic acid and expressed as mg gallic acid equivalents (mg GAE/gFW).

Extraction and quantification of malondialdehyde (MDA)

The extraction and quantification of MDA, an indicator of lipid peroxidation, was performed according to Du and Bramlage (1992) with modifications. Cotyledon samples (0.25 g FW) were homogenized with 3 mL 0,1% (w/v) trichloroacetic acid solution (TCA) and centrifuged at 12000 g for 10 min. Resulting supernatants were used for MDA quantification. Briefly, supernatant aliquot (1 mL) was added with 1 mL of 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid. Resulting mixture was heated in boiling water for 25 min and quickly cooled in an ice bath. Finally, the mixture was centrifuged at 3000 g for 10 min and then the absorbance was read at 440, 532 and 600 nm. MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as μmol Eq MDA/g FW.

Extraction and quantification of photosynthetic pigments

Photosynthetic pigments (chlorophylls and carotenoids) were extracted according to the technique described by Chapelle *et al.* (1992). Cotyledon samples (0.20 mg FW) were homogenized with 2 mL of dimethyl sulfoxide and incubated for 12 h at 45°. Chlorophyll a and b and carotenoids contents were calculated from absorbances at 665, 649 and 480 nm using a spectrophotometer UV-visible (Hitachi U 2800-A, Japan). The tissue concentrations of photosynthetic pigments were determined by the equations of Wellburn (1994).

Total antioxidant capacity assay by ABTS scavenging activity

The antioxidant capacity of soluble phenols present in quinoa plants was determined according to the method described by Re *et al*. (1999). For the study, an $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ($^{\pm}$.02) at 734 nm and equilibrated at 30°C using a spectrophotometer (Biotek ELx808). Different volumes of extract of quinoa seedlings exposed or not to irradiation UV-B were reacted with 200 μl solution of radical $ABTS^{\bullet+}$ at room temperature. After 6 minutes from the start of the reaction, the absorbance was measured at 734 nm.

The percentages of free radical scavenging activity were calculated and expressed as the percentage inhibition of the absorbance of the $ABTS^{\bullet+}$ solution as a function of concentration antioxidants.

Determination of UV-B absorbing compounds

To determine the presence of UV-B absorbent compounds in cotyledon samples without or with irradiation UV-B, Mireki and Teramura (1984) technique was followed. For the extraction of absorbing compounds, 0.20 mg fresh tissue was homogenized with 2 mL of acidified methanol (methanol:water:HCl, 79:20:1) and incubated for 12 h at 45°. Subsequently, prior extraction of vegetal tissue, the absorbance of extracts was measured at 305 nm. The results were expressed as A_{305}/g WF.

Analysis Sun Protection Factor (SPF)

The ability of insoluble phenolics to filter UV-B radiation was measured in quinoa extracts according to the in vitro technique described by Sayre *et al*. (1979). Different concentrations of each extract (0.0125 to 0.2 μg IP/mL) were prepared. The absorbance of solutions was measured at different wavelengths (λ) of UV-B radiation range (290-320 nm) with intervals of 5 nm using a spectrophotometer. The SPF was calculated using an equation according to Borghetti and Knorst (2006).

RESULTS

Soluble (SP) and insoluble phenolics (IP)

The highest content of SP was obtained in seven days-old seedlings irradiated with UV-B for 3 minutes, reaching a value of 2.25 ± 0.13 mg Eq GA/g FW (Fig. 1A). Interestingly, when ten days-old seedlings were irradiated with UV-B, regardless of dose, there were no significant differences between treatments. The values obtained were between 1.45±0.01 and 1.62±0.02 mg Eq GA/gFW.

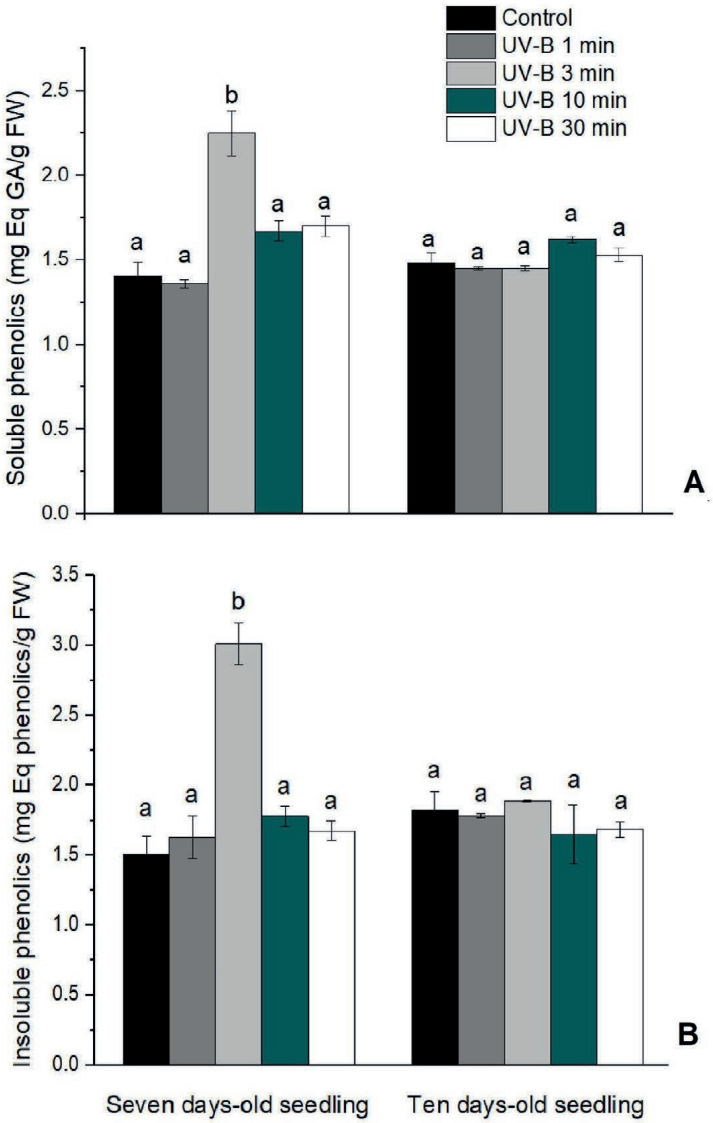

Fig. 1. Effect of UV-B dose on the content of phenolic compounds in cotyledons of seven and ten-day-old quinoa seedlings. A) Soluble phenolics. B) Insoluble phenolics. Different letters indicate statistically significant differences ($p < 0.05$, Fisher's post-test).

Fig. 1. Efecto de la dosis de UV-B sobre el contenido de compuestos fenólicos en cotiledones de plántulas de quinoa de 7 y 10 días. A) Compuestos fenólicos solubles. B) Compuestos fenólicos insoluble. Letras diferentes indican diferencias estadísticamente significativas ($p<0.05$, prueba de Fisher).

On the other hand, a similar trend to IP was observed (Fig. 1B). The maximum concentration of IF was obtained in seven days-old seedlings irradiated with UV-B for 3 minutes, reaching a value of 3.01 ± 0.15 mg Eq phenolic/g FW. This value was significantly higher (67%) than the concentration of IF obtained in the control (1.51 ± 0.12 mg Eq phenolic/g FW). However, the other treatment did not show a significant difference compared with the control seedling without UV-B.

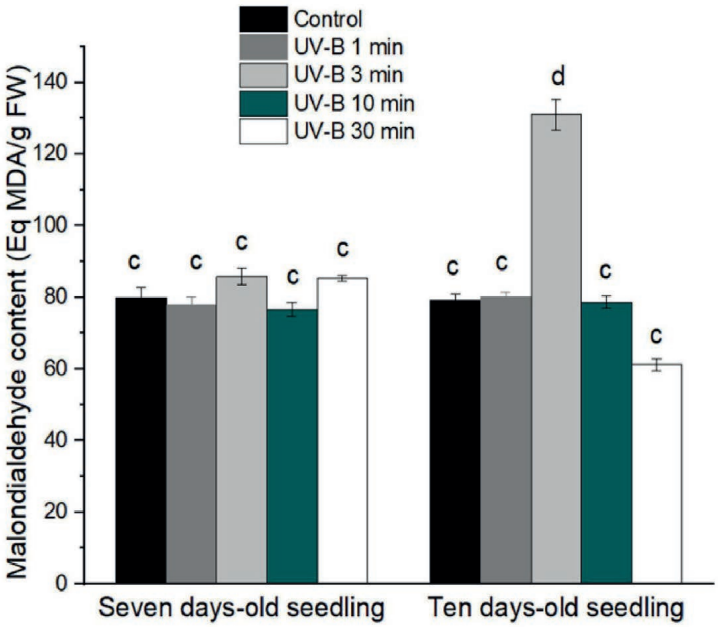

Fig. 2. Content of malondialdehyde (MDA) in cotyledons of 7 and 10 days-old quinoa seedlings exposed to UV-B irradiation. Different letters indicate statistically significant differences ($p < 0.05$, Fisher's post-test).

Fig. 2. Contenido de malondialdehído (MDA) en cotiledones de plántulas de quinoa de 7 y 10 días expuestas a radiación UV-B. Letras diferentes indican diferencias estadísticamente significativas ($p<0.05$, prueba de Fisher).

Malondialdehyde content

Lipid peroxidation produces malondialdehyde (MDA), a secondary derivative of aldehyde, which indicates the presence of oxidative stress in plants under stress conditions. In seven days-old seedlings, no significant difference in MDA values was observed (Fig. 2). However, ten-day-old seedlings irradiated with UV-B for 3 minutes showed a significant increase in MDA content, reaching values of 131 ± 0.05 Eq MDA/g WF($p<0.05$) which was 64% higher than the control one.

Temporal evolution of phenolic compound after UV-B irradiation

According to the results previously obtained, a new treatment was carried out to determine the response time that can induce a higher synthesis of phenolic compounds. Seven days-old seedlings were irradiated with UV-B for 3 minutes at the middle of the photoperiod and after irradiation, seedlings were maintained for 24 h or 48 h under control conditions. The seedlings maintained for 24 h presented the highest concentration of SP (2.49 ± 0.10 mg Eq GA/g WF) (Fig. 3A) while at 48 h the SP content decreased significantly with respect to the control ($p<0,05$).

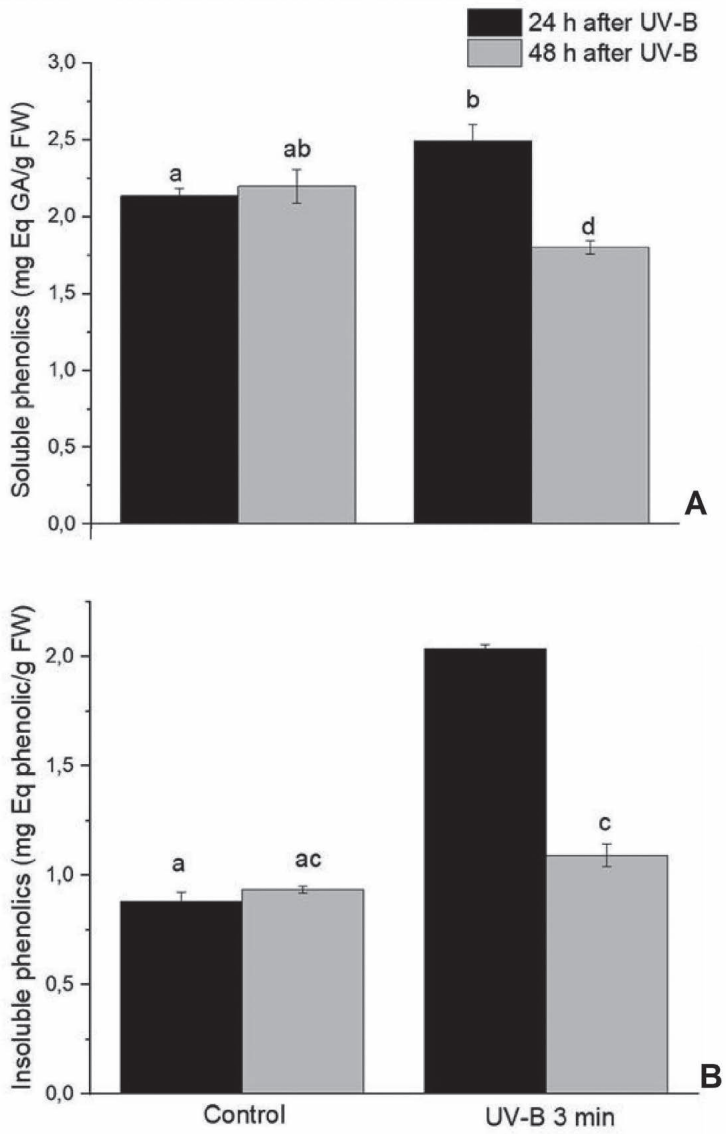

Fig. 3. Temporal evolution of the content of phenolics compounds in cotyledons of seven days-old quinoa seedling after UV-B irradiation. A) Soluble phenolics. B) Insoluble phenolics. Different letters indicate statistically significant differences ($p < 0.05$, Fisher's post-test).

Fig. 3. Evolución temporal del contenido de compuestos fenólicos en cotiledones de plántulas de quinoa de 7 días luego de la irradiación con UV-B. A) Compuestos fenólicos solubles. B) Compuestos fenólicos insolubles. Letras diferentes indican diferencia estadísticamente significativas ($p<0.05$, prueba de Fisher).

Regarding IP content, seedlings 24 h post-irradiation increased the production of these metabolites, reaching values significantly higher (2.04±0.019 mg Eq phenolic/g WF) than those of the control (0.88 ±0.04 mg Eq phenolic/g WF). IP content after 48 h of irradiation decreased 50% approximately compared to 24 h ($p<0.05$) (Fig. 3B).

Table 1. Content of photosynthetic pigments (μg/mL FW) and Chlorophyll a/b ratio in cotyledon of seven days-old seedling exposed to UV-B irradiation. Different letters indicate statistically significant differences ($p < 0.05$, Fisher's post-test).

Tabla 1. Contenido de pigmentos fotosintéticos (μg/mL FW) y relación clorofila a/b en cotiledones de plántulas de siete días expuestas a irradiación UV-B. Las distintas letras indican diferencias estadísticamente significativas ($p < 0,05$, postest de Fisher).

Treatmens	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a/b*	Carotenoids
Control	1561.8 ± 40.9a	429.5 ± 25.5a	3.64 ± 0.24a	418.74 ± 9.36 a
UV-B 3 min	1288.2 ± 47.7b	381.9 ± 18.9b	3.37 ± 0.29a	361.14 ± 8.40 b

Content of photosynthetic pigments

The content of chlorophyll *a*, *b* (Ch *a*, Ch *b*) and carotenoids in the cotyledons of seven days-old seedlings was evaluated. In the plant tissue exposed to 3 minutes of UV-B radiation, it was observed that the concentration of Ch *a*, total and carotenoids decreased significantly with respect to the control (Table 1). While the concentration of chlorophyll b did not show a significant difference with the control.

Antioxidant activity, UV-B absorbing compound, and photoprotection capacity

The extract of seedlings irradiated with UV-B was more active as $ABTS^{\bullet+}$ scavenger than the extract seedlings not irradiated (control), reaching DC_{50} values (concentration of extract necessary for scavenging the 50% of ABTS radicals) of $14^{\pm}$ 0.1 and 17 ± 0.08 μg Eq GA/g SF respectively (Fig. 4).

The content of UV-B absorbing compounds was evaluated in the extracts of seven days-old seedlings, exposed and not exposed to radiation. Interestingly, the concentration of such compounds increased by ~20% in cotyledons after 3 minutes of exposition UV-B, compared to the control, reaching values of 46,7 ± 1,19 A_{305nm} g/FW ($p < 0.05$) (Fig. 4)

On the other hand, the photoprotection capacity was evaluated in extracts (1 mg Eq GA/ ml) of seven days-old seedlings with or without irradiation. Sun protection factor values obtained were higher when the plant was exposed to UV-B radiation. The values reaching were 15.1 and 11. 8 for seedlings with and without irradiation respectively.

DISCUSSION

The plants have developed a variety of responses to adapt to the hazardous UV-B radiation. The most common mechanism of protection against potentially damaging radiation is the biosynthesis of secondary metabolites, such as phenols and flavonoids (Ghasemzadeh *et al*., 2016; Chen *et al*., 2019; Neugart *et al*., 2019). In sensible plants, without protective mechanisms, the

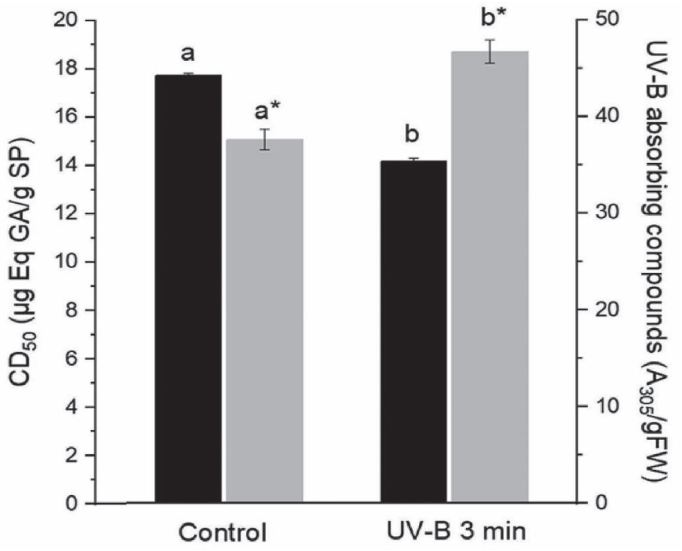

Fig. 4. Antioxidant activity (left axis, black bars) and content of UV-B absorbing compounds (right axis and grey bars) present in cotyledons of seven-day-old quinoa seedlings exposed to UV-B irradiation. Different letters indicate statistically significant differences ($p < 0.05$, Fisher's post-test).

Fig. 4. Actividad antioxidante (eje Izquierdo, barras negras) y contenido de compuestos absorbentes de UV-B (eje derecho, barras grises) presente en cotiledones de plántulas de quinoa de 7 días expuestos a radiación UV-B. Letras diferentes indican diferencias estadísticamente significativas ($p<0.05$, prueba de Fisher).

damages frequently observed are ROS production, decreased photosynthetic pigments, reduced growth, etc. (Chen *et al.*, 2020; García-Caparros *et al.*, 2021). The stressor agent will tend to produce different response phases in the plants, which will depend on genotype, the duration, and intensity of the stress (Schulze *et al.*, 2019). Sun *et al.* (2010) also demonstrated that leaf age was a key factor in the protective response to UV-B radiation and, in relation to quinoa in particular, other authors suggest that this species has different response mechanisms depending on the dose of UV-B received (Hilal *et al.*, 2004; Huarancca Reyes *et al*, 2018; Mariotti *et al.*, 2021; Wittayathanarattana *et al.*, 2022). In this context, the present work evaluated the influence of both factors: UV-B dose and seedling age on the production of secondary metabolites in quinoa. Reifenrath and Müller (2007) showed similar results in two species of *Brassicaceae*, whose younger leaves responded faster against radiation. Thus, in our study, seven-day seedlings could show a higher response to ensure seedling establishment, through the synthesis of protective molecules, such as phenolic compounds (Kumar *et al.*, 2020). Furthermore, in the cotyledons of seven-day-old seedlings, metabolic activity is greater than in older cotyledons, since these could be initiating senescence (around 12-13 days), redistributing C to other growing organs, such as new leaves, to the detriment of the synthesis of primary and secondary metabolites (Ruffino *et al.*, 2008).

Increases in the protective metabolites (soluble and insoluble phenolics) determined in this work are consistent with those presented by other

authors, who have reported that low doses of UV-B radiation significantly increased the content of UV-B absorbing compounds and total phenolics in the peel of harvested lemons (Interdonato *et al*, 2011). In this sense, studies on the biosynthesis of phenolic compounds have shown that UV-B radiation induces the expression of specific genes of the phenylpropanoids pathway, increasing transcription levels and the activity of key enzymes (Reifenrath and Müller, 2007; Rodríguez-Calzada *et al.*, 2019; Rizi *et al.*, 2021; Singh *et al.*, 2023).

Conversely, in this work, the content of phenolic compounds at the highest doses did not show a significant increase (Fig. 2). This fact could indicate that there was an increase in the use of phenolic compounds to eliminate reactive oxygen species (ROS) since the expression of scavenging enzymes, such as peroxidase (POD) and mitochondrial Mn-superoxide dismutase (Mn-SOD) did not is induced by UV-B (Rodríguez-Calzada *et al.*, 2019). The increase in ROS generates lipid peroxidation of cell membranes which produces MDA as the most abundant aldehyde derivatives (Jiang and Zhang, 2002). In the present study, only 10-day-old seedlings irradiated with UV-B for 3 minutes showed high MDA content, which could be due to the lower IP content compared to 7-day-old seedlings (Fig. 1 and 2).

In our study, the UV-B absorbing compounds and IP content were higher in cotyledons of plants irradiated with 3 min UV-B dose, which could produce a screening effect against the penetration of UV-B radiation (Hilal *et al*., 2004, Taiz *et al*., 2018). Moreover, SP, which includes UV-B absorbing compounds, could act as antioxidants protecting cells of ROS (Rozema *et al*., 2002; León-Chang *et al*., 2017). It is known that UV-B radiation increases the flavonoid and other phenolics contents which present screening function (Taiz *et al*., 2018). In this sense, Pérez *et al.* (2015) observed a similar response in leaves of five quinoa varieties that showed an increase in UV-B absorbing compounds. In agreement with this, our results show an increase in the content of UV-B absorbing compounds related, in turn, to a higher antioxidant activity (Fig. 4). Therefore, enriched quinoa extract could have a double function: as sunscreen and antioxidant.

On the other hand, the protective screen effect would also produce a decrease in PAR radiation that would lead to a new organization of the photosynthetic apparatus. Quinoa seedlings showed a higher stacking of thylakoids (shadow-like chloroplasts) in response to UV-B radiation (Hilal *et al*., 2004). Thus, the decrease in photosynthetic pigments observed in this work could be due to acclimation of the photosynthetic apparatus to less PAR radiation. Moreover, it is also important to consider leaf age in relation to its physiological stage, since Rizi *et al.* (2021) found a decrease in chlorophyll content of young leaves of *Salvia verticillata* under UV-B exposure. This suggests that young seedlings could be more sensitive to UV-B and therefore, cotyledons could preferentially direct their metabolism towards the synthesis of protective compounds.

On the other hand, after irradiation, the temporal evolution of protective compounds was evaluated. Our result showed that the highest content of SP and IP were obtained 24 h after irradiation in seven days-old seedlings irradiated with UV-B for 3 minutes (Fig. 3), according to other authors that showed increases in phenolic compounds after 24 h of response time (Wittayathanarattana *et al*., 2022). On the other hand, when the time after irradiation was 48 h, the phenolics content did not increase (Fig. 3). The plant stress response is a dynamic process with several stages. Early stress response during an alarm phase could be characterized by an activation of various inducible stress signaling pathways and enhanced oxidative stress (Rehem *et al*., 2012). However, during recovery time, protective stress compounds are degraded, and a new cellular homeostasis is initiated. Therefore, it could be inferred that quinoa plants exposed to short-term stress showed a brief and rapid acclimation response, restoring their normal physiological metabolism after 48 h.

The sun protection factor (SPF) of seven-day-old seedling extracts was evaluated 24 h after UV-B irradiation. The SPF expresses the efficiency of a compound to absorb in the entire UV range, a higher SPF indicates a stronger photoprotective activity (Radice *et al*., 2016). The extracts obtained from irradiated seedlings showed a high SPF (15.3) according to the classification of SPF by Protection according to European Commission Recommendation [ECR, 2006). An advantage of the use of phytochemicals for protection against the sun is based on their "pluripotent character", as termed by Dinkova-Kostova (2008), which is defined as their ability to counteract the multiple damaging effects of UV radiation.

It should be noted that the SPF value found in non-irradiated plant extracts (SPF value:11) is higher than that reported for other species (Priyanka *et al*., 2018; Álvarez-Gómez *et al*., 2019) and is considered an average SPF value (ECR, 2006). This could be due to quinoa being adapted to grow in high mountain environments under high UV radiation levels, drought, and salinity (Ain *et al*., 2023).

In quinoa plants, more than 130 phytochemical compounds have been identified (Javaid *et al*., 2022; Melini and Melini, 2022). Tang *et al.* (2015) determined in quinoa seeds of three typical colors, the presence of phenolic compounds in free and conjugated forms such as quercetin, kaempferol, ferulic acid, p-coumaric acid, vanillic acid, p- hydroxybenzoic acid, etc. Likewise, the presence of lignin in response to UV-B was shown by Hilal *et al.* (2004). Al-Qabba *et al*. (2020) determined that quinoa sprouts of 6 days-old present a great antioxidant activity. In this sense, the present work represents a first step in the optimization for obtaining extracts rich in phenolic compounds with biological activity for the formulation of natural sunscreens.

On the other hand, our results showed a fresh biomass yield of 0,5 g/cm^2 (cotyledons+hypocotile), which makes the microgreen system an interesting option since it can be carried out in a small space, quickly and

with few resources. This could also be valuable for the development of micro-economies, revaluing quinoa both as a functional food and for its cosmetic/pharmacological use.

CONCLUSION

Quinoa plants present mechanisms of adaptation to UV-B that produce a rapid increase in the synthesis of phenolic compounds, in response to supplementary doses of UV-B. In this search, it was decided to use a microgreen system to obtain seven days-old quinoa seedlings as a resource of extracts with a high content of phenolic compounds. The enrichment of the extracts was achieved by a short dose of UVB radiation (4.12 Wm^{-2} for three minutes). This selection was based on the biotechnological point of view of maintaining an adequate cost/benefit ratio. The evaluated plant extracts showed good sun protection value and antioxidant activity. The methods used to enhance the bioactivities in the quinoa extract were simple, making it an environmentally and economically advantageous process. These results can serve as a starting point for optimization studies through the response surface methodology (RSM). The RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes (Myer *et al*., 2009). It also has important application in the design, development, and formulation of new products. In this sense, the application of RSM to our data will allow us to adjust the variables studied to obtain the best possible responses. One of the most positive aspects of using RSM is that it can predict response patterns and thus reduce the number of experiments needed to obtain reliable results, which saves time and resources.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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