JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2022, VOL. 5(4), 297-308

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

An abiotic UV-B stress on Brassicaceae seeds increased their phytochemical content on 7-days sprouts

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ARTICLE INFO

Original Article

Article history:
Received 22 June 2022
Revised 27 September 2022
Accepted 11 October 2022
Available online 10 November 2022

Keywords:
Antioxidants
Bioactive compounds
Brassica
Cruciferous
Germination

DOI: 10.22077/jhpr.2022.5406.1278
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: Cruciferous sprouts in their early stages of development are very suitable foods against degenerative diseases due to their high content of health promoting compounds. The application of UV-B can act as an elicitor of these compounds. The objective was to study the effect of a UV-B treatment to different seed varieties and its remnant effects after germination as sprouts. **Research method:** *Brassicaceae* seeds selected (radish, rocket, white mustard, and tatsoi) were treated under 20 kJ m^{-2} UV-B 24 h before sowing, while no UV radiation was used as control (CTRL). After 7 days, sprouts were harvested, frozen, and freeze-dried until their bioactive content was analysed. **Findings**: Results showed that UV-B enhanced by ~20% the biosynthesis of phenolic compounds in sprouts, and hence, their total antioxidant capacity. Furthermore, this UV effect was also appreciated after 7 germination days, and it was increased by ~38% regarding CTRL, especially on rocket and mustard sprouts. In conclusion, an abiotic UV-B stress treatment to seeds can be an interesting tool to improve the bioactive compounds content of young plants, although the intrinsic mechanisms involved should be further investigated. **Research limitations:** The use of these new technologies, such as UV-B, is costly and must be applied following appropriate safety measures to avoid possible irradiation damage. **Originality/Value:** The analysis of the remnant effect of the UV-B before seeds sprouting has not been yet studied and its use could result in a beneficial effect on the germination and biosynthesis of phytochemicals in young plants.

INTRODUCTION

Sprouts emerge as a new way of consuming vegetables with a high content of bioactive compounds. A sprout can be defined as 'the product obtained from the germination of seeds and their development in water or other medium, being harvested before the development of true leaves with the intention of being eaten whole, including the seed' (Di Gioia et al., 2017). The most common are those belonging to the *Brassicaceae* family (e.g. broccoli, rocket, mizuna, kale, Chinese cabbage, mustard, kale, tatsoi, radish), *Asteraceae* (e.g. lettuce, endive, escarole), *Apiaceae* (e.g. fennel, carrot, celery), *Amarillydaceae* (e.g. garlic, onion), *Amaranthacea* (e.g. spinach, beetroot), and *Cucurbitaceae* (e.g. melon, cucumber) (Di Gioia et al., 2015). They are presented as an interesting alternative as they contain large amounts of health-promoting compounds in a small amount of vegetable, thus having a high phytochemicals concentration (Shubha et al., 2019).

Therefore, sprouts are the ideal product for current consumption trends since, when consumed in the early vegetative growth phase, the content of glucosinolates, phenols, selenium compounds, proteins, amino acids, and vitamins is higher than in the adult plant (Shubha et al., 2019). Another great benefit of sprouts is that they are easy to consume and can be eaten on their own in a single serving or supplemented to salads. Accordingly, cruciferous sprouts, as an important source of glucosinolates and isothiocyanates, in their early stages of development are very suitable foods against degenerative diseases due to their high content of health promoting compounds.

An abiotic stress refers to environmental factors that alter the physiological and metabolic processes of plants (Taiz & Zeiger, 2018). In the case of sprouts, some of the main abiotic stresses that have major importance and technical feasibility of implementation are UV light during germination and postharvest.

UV-B light is light in the wavelength range 280-315 nm. The UV-B photoreceptor is called UV RESISTANCE LOCUS8 (UVR8) and regulates the transcription of more than 70 genes, participating in flavonoid synthesis, DNA repair and reduction of oxidative stress damage. This photoreceptor also regulates morphological processes such as cotyledon opening, hypocotyl extension, phototropism, leaf expansion and stem extension (Jenkins, 2014). This photoreceptor also plays a role in HY5 gene expression, causing overexposure to UV-B to result in morphological problems such as poor response to hypocotyl growth (Cloix et al., 2012).

In previous studies, exposing mung bean seeds to UV-B light during germination resulted in reduced chlorophyll-a and -b activities and apical shoot growth 40 days after germination, increasing plant dry and fresh weight, and stem diameter (Li et al., 2014). In another crucifer such as kale, it was found that applying a periodically dose of UV-B during seeds germination (with a total applied of 15 kJ m^{-2}) stimulated the biosynthesis of glucosinolates such as glucoiberin, glucoraphanin, or glucobrassicin (Castillejo et al., 2021). Thus, the application of UV-B can act as an elicitor of these compounds, at least during the germination and after harvesting during the shelf-life period.

However, what happen if this stress is applied directly to the seed before sowing? As response to this question, the objective of the present study was to evaluate the effect of a UV-B treatment to different seed varieties and its remnant effect after their germination as sprouts into their bioactive content.

MATERIALS AND METHODS

Seed selection

Brassicaceae seeds selected were radish (*Raphanus sativus*), rocket (*Eruca vesicaria*), white mustard (*Sinapis alba*), and tatsoi (*Brassica rapa*). All seeds were provided by Intersemillas S.A. (Valencia, Spain).

UV-B treatment

The UV treatments were performed in a reflective stainless-steel chamber. The radiation chamber, which is fully described by Castillejo et al. (2021), which was equipped with 6 UV-B unfiltered emitting lamps (TL 40W/01 RS; Philips, Eindhoven, The Netherlands). The seeds were placed between the two lines of lamps at 17.5 cm above and below. The applied UV-B intensity of 8.10 ± 0.35 W m^{-2} was calculated as the mean of 20 readings on each side of the net using a LP 471 UVB (Delta OHM, Italy) radiometer. The applied dose was previously determined to avoid UV damage to the cruciferous seeds, according to preliminary tests and our own experience. The treatments and doses were CTRL: No UV treatment was used as control. UV-B: the seeds received an effective dose of 20 kJ m^{-2} UV-B. After UV treatments, seeds were kept at room temperature for 24 h and afterwards their bioactive content was assessed. The germination process also started after this time.

Germination process and harvest

Two g per tray of UV-B treated and untreated seeds were rinsed twice (1 min each one). Subsequently, the seeds were sown in a laminar flow hood. Seeds were sown on polypropylene trays (17 × 12 × 5 cm), using a layer of filter paper as support. To ensure a high relative humidity (RH), we covered the trays with a 40 µm biaxial oriented polypropylene film (PDS Group; Murcia, Spain). Selected seeds were germinated under darkness conditions for 7 days at 20°C and 80-90% RH in a growth chamber (Sanyo MLR-350 H, Osaka, Japan). After this time, the sprouts were harvested and washed for 1 min with a sodium hypochlorite solution (150 ppm; 5ºC; pH=6.5) and then rinsed in water for 1 min at 5ºC. A 2 min dewatering process on a paper filter sheet was accomplished. Thereafter, 30 sprouts were photographed next to a rule (3 replicates of 10 sprouts; n = 30) to measure their length using the ImageJ program (Wayne Rasband, Maryland, USA). Sprouts were subsequently frozen into sealed plastic bags and freeze-dried for further analysis.

Phenolic compounds analysis

The extraction was developed following the method described by Martínez-Zamora et al. (2021a), freeze-dried seeds or sprouts (50 mg) were placed in plastic tubes with 6 mL methanol/H_2O (80:20, v/v). For each treatment, nine replicates (n = 9) were extracted in an orbital shaker where the samples were shaken for 1 h, at 4°C, in darkness. The supernatant was collected after a centrifugation at 3,220 × g for 10 min at 4 °C to measure total phenolic content, total flavonoid content, and total antioxidant capacity.

The method of Singleton and Rossi (1965) with some modifications was performed to determine the total phenolic content, as described by Martínez-Zamora et al. (2021a). For that, 0.019 mL of the sample extract was mixed with 0.029 mL of 1 mol L^{-1} Folin–Ciocalteu reagent and 0.192 mL of 0.4% Na_2CO_3 and 2% NaOH. The absorbance was measured at 750 nm using a microplate reader (Tecan Infinite M200; Tecan Trading AG, Männedorf, Switzerland) after 1 h of incubation under darkness conditions. The total phenolic content was expressed as mg chlorogenic acid equivalents (CAE) g^{-1} dry weight (DW) using a chlorogenic acid standard curve; $R^2 > 0.99$.

Using the same methanolic extract, total flavonoid content was determined by following the method described by Hamed et al. (2019). Briefly, 0.030 mL of extract was mixed with 0.080 mL of aluminium chloride (20 g L^{-1}). The samples were shaken for 30 s and then incubated in darkness for 1 h. The reaction absorbance was measured at 415 nm. Total flavonoid content was expressed as mg rutin equivalents (RE) g^{-1} of DW using a rutin standard curve; $R^2 > 0.99$.

Total antioxidant capacity

The total antioxidant capacity was analysed using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) method (Castillejo et al., 2017). Thus, 0.194 mL of DPPH• (700 µM) solution was added to 0.021 mL of sprout or seed extract. After 30 min incubation in darkness, absorbance was measured at 515 nm. The total antioxidant capacity was expressed as mg trolox equivalents (TE) g^{-1} DW using a trolox standard curve; $R^2 > 0.99$.

Statistical analysis

The independent variable or factor was the UV treatment, which was subjected to an analysis of variance (ANOVA) using the statistic software Statgraphics Plus software (v. 5.1. Statpoint Technologies. Inc. Warrenton. VA. USA). $P < 0.05$ was assessed as statistically significant, and Tukey's multiple range tests was used to separate means.

RESULTS AND DISCUSSION

Sprout growth

After 7 germination days at 20ºC in darkness, UV-B-treated and untreated seeds produced radish, rocket, mustard, and tatsoi sprouts. The longest sprouts were mustard (~7.91 cm), followed by radish (~7.13 cm), rocket (~4.14 cm), and tatsoi (~3.05 cm), as shown in Table 1. As observed, the only crops affected by the UV-B treatment previously applied to the seeds were tatsoi. The hypocotyl length of such sprouts increased by ~48%, which incremented the total sprouts length by ~51% in comparison with CTRL sprouts obtained from untreated tatsoi seeds.

In contrast, the physiological growth of the other three studied species was not affected by the UV-B pre-treatment applied to their seeds.

In addition, Peykarestan et al. (2012) showed as seed germination after UV irradiation (from 220 to 400 nm) was increased while decreasing irradiation dose for both Sayad and Derakhshan bean seeds. In addition, the final germination percentage was non-significantly affected in Sayad with all irradiation doses. As shown by these authors, shoot length was decreased in both kind of seeds after all UV doses compared to their control (non-irradiated), nevertheless, we have to take into account that applied doses by Peykarestan et al. (2012) were higher than the ones applied in the present study, which can explain that we did not obtain significant differences between CTRL and UV-B treatments regarding sprout growth. As a matter of fact, recently in our previous publications, we observed as low doses of UV-B applied during sprout growth (from the third to the tenth day of growing), did not affect to the hypocotyl and sprout length in kale (Castillejo et al., 2021) and red cabbage sprouts (Martínez-Zamora et al., 2021b). This behaviour could be explained by the fact that plants under UV lighting can develop specific responses, which results in an increase of the length of the hypocotyls (Escobar-Bravo et al., 2017).

Table 1. Sprout characterization after 7 germination days at 20 ºC in darkness from control (CTRL) or UV-B-treated (20 kJ m^{-2}) radish, rocket, mustard, and tatsoi seeds from Brassicaceae family

	Treatment	Hypocotyl length (cm)	Radical length (cm)	Sprout length (cm)
Radish	CTRL	4.07 ± 0.29	3.06 ± 0.12	7.13 ± 0.22
	UV-B	4.26 ± 0.27	2.95 ± 0.34	7.21 ± 0.58
Rocket	CTRL	2.32 ± 0.23	1.83 ± 0.57	4.14 ± 0.42
	UV-B	2.16 ± 0.14	1.30 ± 0.50	3.46 ± 0.62
Mustard	CTRL	4.01 ± 0.25	3.50 ± 0.41	7.91 ± 0.88
	UV-B	4.19 ± 0.23	3.88 ± 0.49	8.07 ± 0.75
Tatsoi	CTRL	1.47 ± 0.14^{B}	1.58 ± 0.38	3.05 ± 0.51^{B}
	UV-B	2.18 ± 0.11^{A}	2.43 ± 0.55	4.61 ± 0.63^{A}

Different letters (A, B) denote significant differences among treatments at $p < 0.05$ according to Tukey's test ($n = 30$).

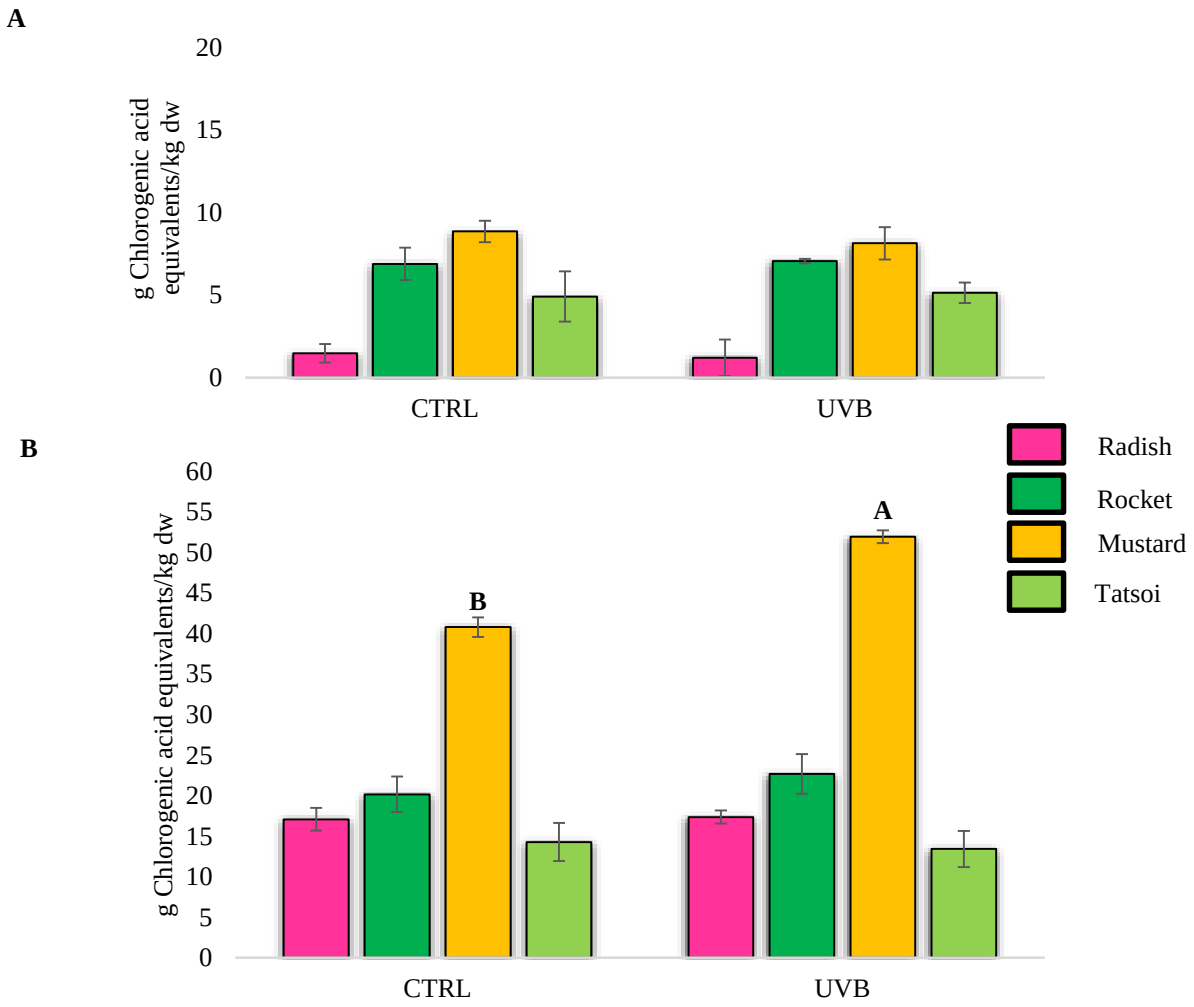

Fig. 1. Total phenolic content of control (CTRL) or UV-B-treated (20 kJ m^{-2}) radish (pink), rocket (dark green), mustard (yellow), and tatsoi (light green) seeds (**A**) and its sprouts after 7 germination days at 20 ºC (**B**). Different letters (A, B) denote significant differences among treatments at $p < 0.05$ according to Tukey's test ($n = 9$).

Total phenolic content and flavonoid biosynthesis

Figure 1 shows the total phenolic content of radish, rocket, mustard, and tatsoi seeds (A) and their sprouts (B). As observed, from a general point of view in both treatments (CTRL and UVB) the species with the highest total phenolic content in their seeds were mustard, rocket, and tatsoi, followed by radish, in this order. Nevertheless, the total phenolic content of their sprouts followed a different behaviour pattern. In this sense, mustard sprouts showed the highest content in these bio-compounds and was followed by rocket, radish, and tatsoi. In fact, mustard sprouts preserved the remnant effect of UVB treatment after sprouting, which was shown by an increase of ~27 % in the total phenolic content of UVB mustard sprouts compared to CTRL mustard sprouts,

Furthermore, comparing Figure 1A and Figure 1B, an exponential increase of the phenolic content of all the studied species can be observed. During germination, mustard, rocket, tatsoi, and radish increased 4-fold, 2-fold, 10-fold their bioactive compounds in comparison with obtained values in their own seeds, respectively.

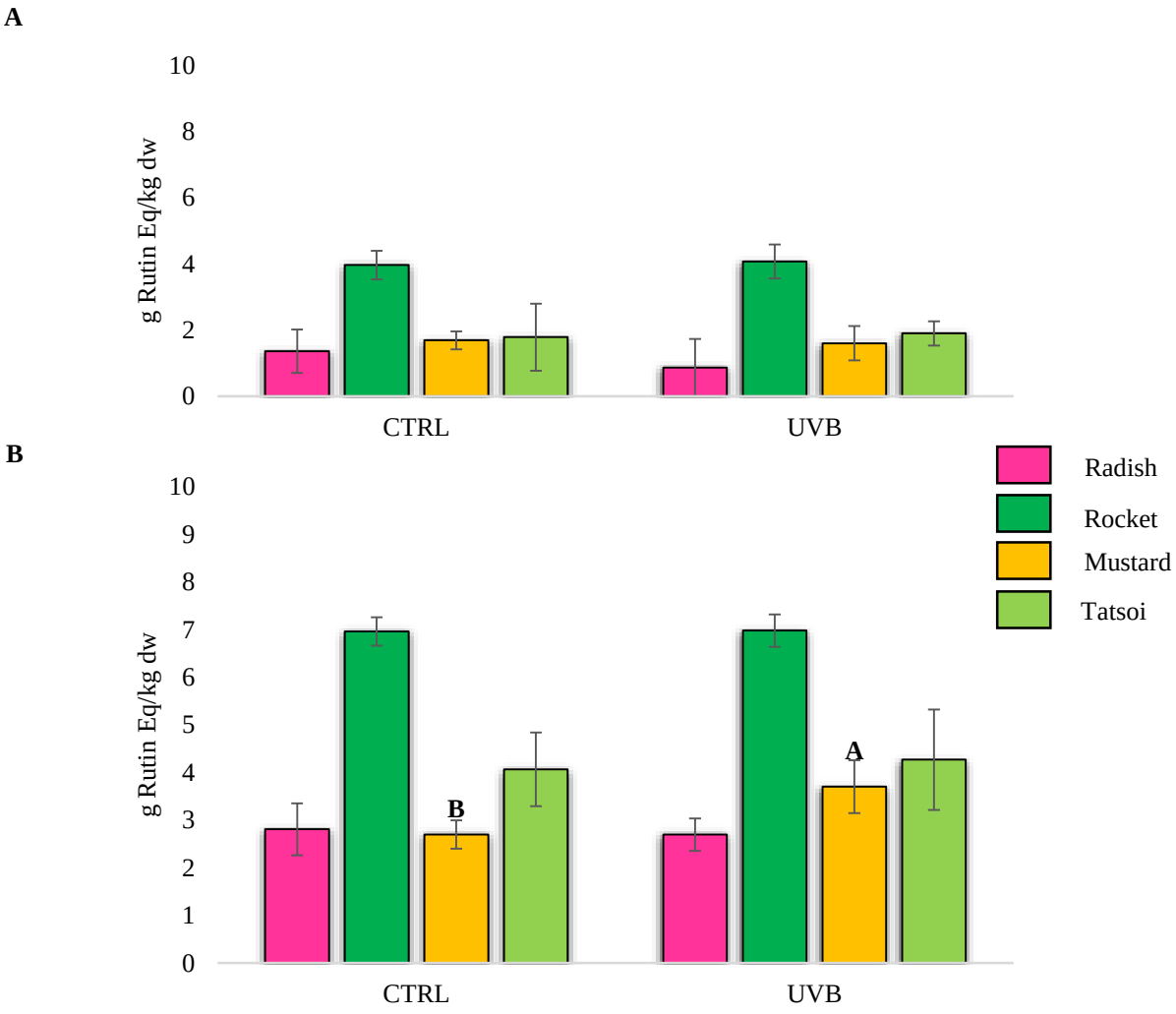

Fig. 2. Total flavonoid content of control (CTRL) or UV-B-treated (20 kJ m^{-2}) radish (pink), rocket (dark green), mustard (yellow), and tatsoi (light green) seeds (**A**) and its sprouts after 7 germination days at 20 ºC (**B**). Different letters (A, B) denote significant differences among treatments at $p < 0.05$ according to Tukey's test ($n = 9$).

This fact is also repeated in Figure 2, where the total flavonoid content of radish, rocket, mustard, and tatsoi seeds (A) and their sprouts (B) is shown. In this case, the richest variety in these compounds was rocket, followed by tatsoi, mustard, and radish, which was reported as in seeds as in sprouts. Although the increase of flavonoids was not as exponential as the total phenolic content, the flavonoid biosynthesis increased twice during the germination period of radish and rocket seeds. Following a different tendency, radish and tatsoi sprouts (both CTRL and UVB) increased their flavonoids content from the seeds and after 7-germination days by twice, while rocket sprouts increased their flavonoid content by ~75 % (both CTRL and UVB) compared to their seeds, which can be appreciated when we compared Figure 2A (seeds) and Figure 2B (7-days sprouts).

With regards to the application of UV-B to the seeds before their sowing, only mustard sprouts showed a remnant effect of such treatment. In this sense, UV-B treated mustard seeds developed into 7-days mustard sprouts with ~27 % higher content of total phenolic content compared to CTRL sprouts, from untreated mustard seeds which, as a mean of all the studied species showed an increase by ~20 %. Furthermore, this behaviour was repeated when we studied the flavonoid biosynthesis, because the flavonoid content of mustard sprouts from UV-B treated seeds increased by ~37 % compared to CTRL mustard sprouts. Nevertheless, this tendency was not followed by the remaining *Brassicaceae* sprouts.

These increases in the concentration of phenolics and flavonoids compounds could be due to the stimuli produced by low doses of UV-B in the phenolic biosynthesis chain that previous authors have demonstrated (Schreiner et al., 2014). A new research field have been opened after the discovery of the influence of abiotic stresses into the regulation of the biosynthesis of nutraceuticals. As such, low and ecological UV-B doses can trigger changes in the secondary plant metabolism, from which phenolics, carotenoids, glucosinolates, and isothiocyanates are derived. Hence the application of this emerging technology has demonstrated to be useful to enhance the biosynthesis of such compounds throughout these metabolic routes in both cases: during the germination of *Brassicaceae* sprouts (Castillejo et al., 2021; Martínez-Zamora et al., 2021b) and after harvesting sprouts of the same family (Martínez-Zamora et al., 2021c). The remnant effect of UV-B during *Brassicaceae* sprout germination may be also a useful tool to treat seeds before sowing, as our results shows, although no previous research has been found in this specific item. In addition, other emerging technologies applied in *Brassicaceae* seeds before sowing, as ultrasounds has also demonstrated to be effective to enhance the biosynthesis of some phytochemicals as sulforaphane, as in rocket seeds and in their sprouts, showing its remnant effect (Martínez-Zamora et al., 2022).

Total antioxidant capacity

Figure 3 shows the total antioxidant capacity of radish, rocket, mustard, and tatsoi seeds (A) and their sprouts (B). However, no differences were found on the different seeds treated with UV-B compared to CTRL treatment of the same species. Following a similar tendency to total phenolic content, mustard seeds (both CTRL and UVB) had the highest antioxidant activity with 3.6 g ET per kg DW, followed by rocket (1.8 g ET per kg DW), tatsoi, and radish seeds (0.8 g ET per kg DW), but these two last ones had no differences between both.

By contrast, total antioxidant activity of the sprouts obtained from those seeds followed a different development as described in Figure 3. Rocket sprouts reported 7.3 g ET per kg DW, while the rest of studied species obtained 6.2 g ET per kg DW (mustard sprouts), 5.5 g ET per kg DW (radish sprouts), and 4.4 g ET per kg DW (tatsoi sprouts). In this case, the highest antioxidant capacity is related to the highest content in flavonoids that rocket presents, which have also demonstrated their potential regarding the antioxidant ability (Falcone Ferreyra et al., 2012).

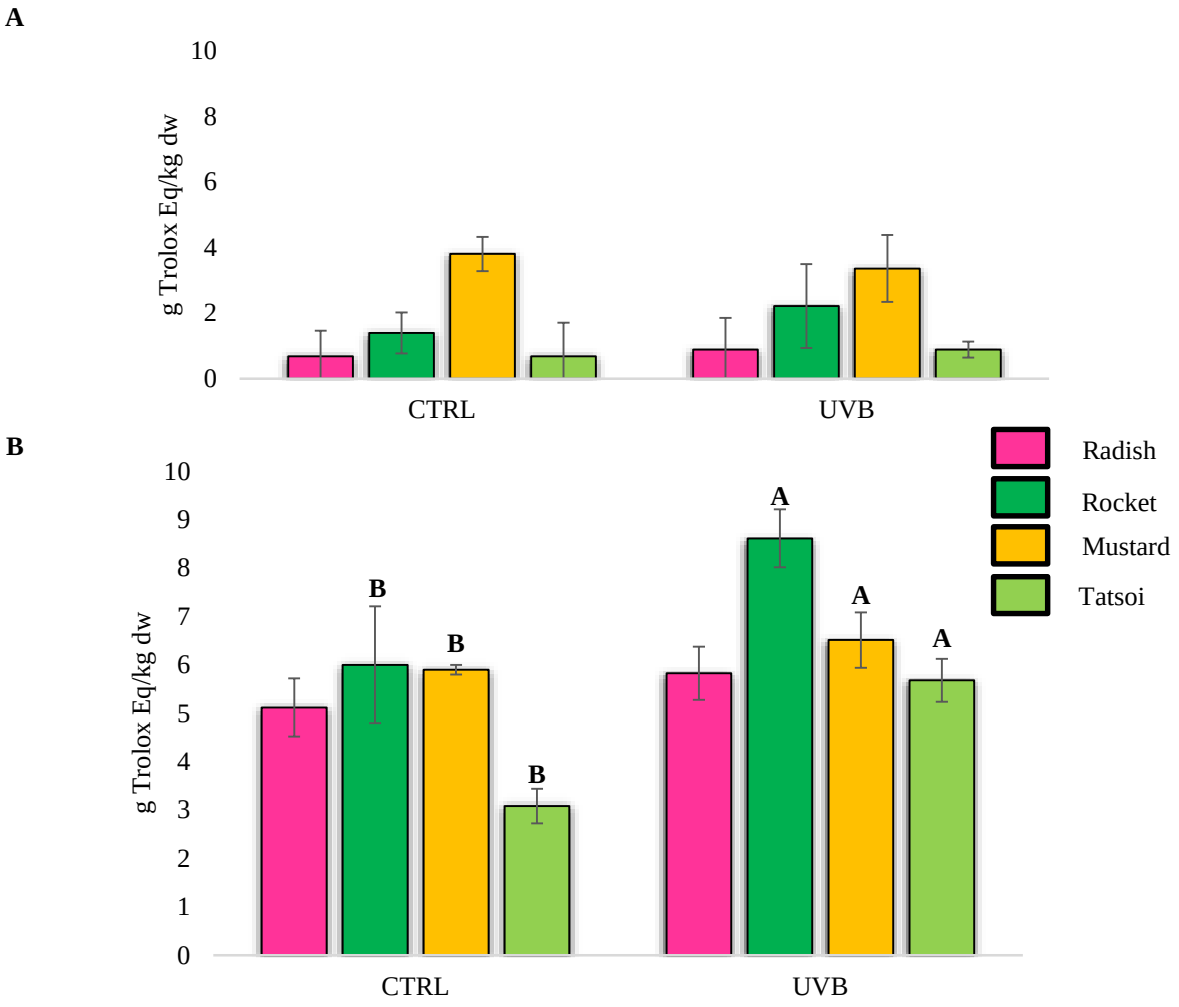

Fig. 3. Total antioxidant activity measured by DPPH of control (CTRL) or UV-B-treated (20 kJ m^{-2}) radish (pink), rocket (dark green), mustard (yellow), and tatsoi (light green) seeds (**A**) and its sprouts after 7 germination days at 20 ºC (**B**). Different letters (A, B) denote significant differences among treatments at $p < 0.05$ according to Tukey's test ($n = 9$).

As Furthermore, the UV-B pre-treatment showed a remnant effect after germination, which showed an important increase in the total antioxidant capacity by ~44 % for rocket sprouts, ~10 % for mustard sprouts, ~84 % for tatsoi sprouts, and ~14 % for radish sprouts.

Main precursors of the antioxidant activity, it was to be expected that an increase in the concentration of the compounds would be related to an increase of this health effect. For that reason, although no previous research has been found in relation to this behaviour, if a remnant effect of UV-B has been appreciated after *Brassicaceae* germination of treated seeds in comparison with untreated seeds, this increase in the total antioxidant capacity may be an interesting healthy effect to pursue furthermore, when the industry is focused on the development of healthier fruit and vegetables to satisfy the needs of a more health-conscious population and to prevent cellular oxidation as a major cause of incipient chronic diseases.

Regarding the obtained results, it is known that the abiotic stress produced UV-B induces genes involved in the phenylpropanoid biosynthesis pathway (Jenkins, 2009; Morales et al., 2010). In fact, when UV light is absorbed, bioactive compounds as flavonoids and phenolic acids responsible of the antioxidant capacity of the plant, act avoiding UV penetration into the vegetal tissue to protect it from damage caused by ROS (Jenkins, 2009; Morales et al., 2010; Solovchenko & Merzlyak, 2008). In fact, UV-B can induce physiological changes and

stimulate defence by the increase of these bioactive compounds in plants. Therefore, UV-B may trigger the synthesis of phytochemicals in young plants and vegetables during sprouting, even when it is applied before sowing.

CONCLUSION

The application of 20 kJ m^{-2} UV-B in seeds did not immediately improve the phytochemical content after its application, which was observed in the results obtained after seeds analysis. Nevertheless, after 7 germination days at 20 ºC and 85 % RH in darkness, a pre-treatment with 20 kJm^{-2} UV-B to seeds improved phenolics accumulation by ~20 %, as mean values obtained from the different studied Brassicaceae species, and which was a related to an increase of ~38 % of the total antioxidant capacity in mustard and rocket species, which may demonstrate the beneficial effect of a UV-B pre-treatment to seeds before germination, as an interesting tool to improve the bioactive compounds content of young plants. Nevertheless, these treatments must be optimized, and further studies must be developed to ensure the benefits of the application of this technology.

Author Contributions

Conceptualization and methodology: Francisco Artés–Hernández (F.A.H) and Lorena Martínez-Zamora (L.M.Z); performed the experiments: Noelia Castillejo (N.C) and L.M.Z..; investigation: F.A.H., L.M.Z., Perla A. Gómez (P.A.G.), Francisco Artés (F.A.), and N.C.; software: L.M.Z.; validation: F.A.H., L.M.Z., P.A.G., F.A., and N.C.; resources: F.A.H.; data curation: N.C.; writing—original draft preparation + review and editing: F.A.H., L.M.Z. and N.C.; visualization: F.A.H.; supervision: F.A.H.; project administration: F.A.H.; funding acquisition: F.A.H.

Funding

This research was funded by the Autonomous Community of the Region of Murcia, grant number 20849/PI/18, through the grant call for projects for the development of scientific and technical research by competitive groups, included in the Regional Programme for the Promotion of Scientific and Technical Research (Action Plan 2018) of the Seneca Foundation—Science and Technology Agency of the Region of Murcia (Spain).

Conflict of interest

The authors declare that there are not conflicts of interest.

Acknowledgments

Noelia Castillejo contract was funded by a predoctoral grant (FPU16/04763) from the Spanish Ministry of Education. Lorena Martínez-Zamora contract has been financed by the Programme for the Re-qualification of the Spanish University System during the three-year period 2021-2023, Margarita Salas modality for the University of Murcia.

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