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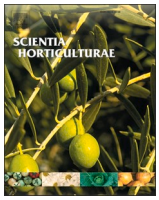

Research Paper

Light Use Efficiency of broccoli (*Brassica oleracea* var. *italica* Plenck) and rocket (*Eruca sativa* L.) during the initial plant growth stages

Donata Arena [a], Hajer Ben Ammar [a,*], Nikola Major [b], Tvrtko Karlo Kovačević [b], Smiljana Goreta Ban [b], Simone Treccarichi [a], Roberto Lo Scalzo [c], Ferdinando Branca [a]

[a] Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5 95123 Catania, Italy
[b] Institute of Agriculture and Tourism 52440 Poreč, Croatia
[c] CREA Research Centre for Engineering and Agro-Food Processing, via G. Venezian 26 20133 Milano, Italy

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ABSTRACT

The solar radiation (SR) is one of the key factors for plant adaptation, and of its growth and development, and for several horticultural crops is important for reaching good yield and produce quality. The SR vary in Europe from 2189 to 517 kwh m^{-2} from Sicily to North cape. Sprouts, microgreens, and baby-leaves, recognized as novel foods due to their high nutraceutical value, have attracted significant attention in the market. The novel foods production could be affected by solar radiation especially when it is carried out in greenhouse in several countries and along their latitudes. Our study aimed to investigate how solar radiation affects the growth of these plants by conducting a thorough analysis of their morphometric characteristics and biochemical profiles. Two different genotypes of commercial rocket, namely *Eruca sativa* L. var. *darkita* and var. *lobata*, along with the Sicilian Black sprouting broccoli (*Brassica oleracea* var. *italica* Plenck, Broccolo nero), were examined at different initial plant growth stages, sprouts, microgreens, and baby-leaves. The experimental trial was carried out in Sicily (Italy) utilizing black shade nets with varying levels of solar radiation (SR100, SR60, SR40). The harvested plantlets were analysed for their main morphometric traits, as sprout weight, hypocotyl length, cotyledon dimensions, in addition to the dimensions of the first true leaf for microgreens, and stem length for baby-leaves. Several biochemical parameters, such as total phenolic content (TPC), total flavonoid content (TFC), and several antioxidant assays including DPPH (2,2-diphenyl-1-picrylhydrazyl), ORAC (oxygen radical absorbance capacity), and FRAP (ferric reducing antioxidant power) were determined. The total glucosinolate content, the sucrose, fructose, fructooligosaccharides (FOS), and total sugars amount were detected. We observed significant variations in the sugar content, particularly under reduced solar radiation and during the baby-leaves stage, characterized by decreased FOS levels and simultaneous increases in sucrose, glucose, and fructose. Moreover, antioxidant capacity showed a progressive enhancement from sprouts to baby-leaves. FRAP and DPPH capacity were significantly affected by the genotype. Interestingly, TFC was the most influenced by SR100, particularly in microgreens. This expanded knowledge base not only advances scientific understanding of these novel foods but also provides substantial adaptation to the different solar radiation conditions in different latitude of the world, permitting to individuate the genotypes more efficient in relation to the solar radiation available.

1. Introduction

In recent decades, there has been a heightened consumer interest in functional foods of plant origin as individuals seek balanced and nutritious diets. In response, the food supply chain has introduced innovative products such as sprouts, microgreens, and baby leaf salads (Galieni et al., 2020). According to Commission Regulations (EU) 208/2013, sprouts are defined as "products obtained from the germination of seeds, collected before the development of true leaves and with only cotyledon disclosure" while microgreens (harvested with one true leaf), and baby-leaves, according to Commission Regulation (EU) 752/2014, are plantlets harvested with 3–4 true leaves up to 8 true leaves (Di Gioia et al., 2017). The development of novel foods that enhance local agro biodiversity has emerged as a prominent strategy to meet evolving

* Corresponding author.
E-mail addresses: hejer.biologie@gmail.com, hejer.benammar@etudiant-fst.utm.tn (H. Ben Ammar).

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dietary preferences while reducing environmental impact. Novel foods hold a significant position within contemporary dietary paradigms, offering a progressive approach to nutrition that transcends traditional dietary choices (Ragusa et al., 2017).

The *Brassicaceae* family encompasses a diverse array of cruciferous vegetables, including broccoli, kale, cauliflower, cabbage, kohlrabi, arugula, turnips, mustard greens, radishes, various species of wild rocket valued for their edible leaves, stems, or flower heads (Branca and Cartea, 2010). The rich diversity is evident, and it is related to the old domestication process occurred in the Mediterranean basin extended by the crop wild relatives (CWRs) (Maggioni et al., 2014). In recent times, the use of biostimulant has become prominent for optimizing the characteristics of Brassica crops. The targeted application of these biostimulant has demonstrated the ability to elicit a physiological response, accelerating growth and enhancing overall plant quality, thereby contributing to the optimization of agronomic yield (Bulgari et al., 2015; Arena et al., 2024). This phylogenetic flexibility has enabled extensive selective breeding, resulting in the development of a wide array of cultivars tailored to various culinary preferences and adaptable to diverse climatic conditions (Salgotra and Chauhan, 2023).

As a model organism in plant science research, *Brassica oleracea* L. remains a subject of sustained scientific interest, contributing valuable insights into essential genetic (Ben Ammar et al., 2021) and physiological processes (Yan et al., 2023). *Brassica oleracea* L. is notable for its distinctive biochemical profile, characterized by an abundance of bioactive compounds with antioxidant properties, such as glucosinolates, polyphenols, ascorbic acid, and vitamins (Bhandari and Kwak, 2015; Borowski et al., 2008; Di Bella et al., 2022a). Similarly, *Eruca sativa*, commonly known as rocket salad, has also been the focus of numerous studies highlighting its high content of bioactive compounds, including glucosinolates, carotenoids, vitamin C, fibers, and flavonoids (Sun-Ju Kim and Gensho, 2006; Barillari et al., 2005).

Glucosinolates are renowned for their well-established antioxidant and anti-inflammatory properties, as well as their potential to mitigate chronic degenerative human diseases (Hayes et al., 2008). In Brassica species, another class of bioactive compounds is polyphenols, which can be categorized into two major groups based on their chemical structure: flavonoids (encompassing flavanols, flavones, anthocyanidins, isoflavones) and non-flavonoids (including phenolic acids, hydroxycinnamates, gentobiosides) (Quideau et al., 2011). Hydroxycinnamic derivatives and flavonoids are the primary compounds present in Brassica crops. It's worth noting that alkaloids, flavonoids, diterpenes, coumarins, polyphenols, and tannins, as demonstrated by numerous studies, are also present in *Eruca sativa* (Sadiq et al., 2014).

Several studies have shown that the antioxidant properties of polyphenols significantly affect various stages of cancer progression (Tapiero et al., 2002). The agronomic traits and the composition and quantity of bioactive compounds can be altered by various factors, including plant parts, developmental stages, as well as external factors like environmental stresses and biological influences (Jin et al., 2009; Soengas et al., 2018; Di Bella et al., 2022b). The main abiotic stresses encompass changes in light exposure, temperature fluctuations, periods of drought, and elevated salinity levels. In response to unfavorable environmental conditions, Brassica crops frequently enhance their antioxidant defence mechanisms to mitigate the harmful impacts of oxidative stress (He et al., 2018; Podsędek, 2007).

Light is an important environmental factor significantly influencing the morphological and biochemical characteristics of broccoli (*Brassica oleracea* L. var. *italica*) (Guo, et al., 2023). Broccoli plants exhibit distinct adaptations in response to varying solar radiation conditions, a phenomenon known as photomorphogenesis (Zhang et al., 2021). Biochemically, exposure to solar radiation has a relevant impact on the accumulation of pigments and bioactive compounds in Brassica crops. Adequate light levels promote increased chlorophyll content, facilitating efficient photosynthesis. Solar radiation stimulates the biosynthesis of carotenoids, such as beta-carotene, lutein, and zeaxanthin, as well as anthocyanins. This not only enhances the visual appeal of broccoli but also contributes to its nutritional value (Crupi et al., 2023; Elmardy et al., 2021; Lefsrud et al., 2007). Several studies have shown the effect of solar radiation on plants, demonstrating its important role in the growth, productivity and the quality of the plant. However, the high or lower solar radiation could be representing an abiotic stress for the plants, causing cellular damage and the formation of the reactive oxygen species (ROS) (Roeber et al., 2021; Ferrante and Mariani, 2018). Consequently, plants activate different mechanisms as morphology modification of the plant, increase the biosynthesis of antioxidant compounds, as phenolic compounds and so activate various antioxidant pathways (Nenadis et al., 2015). Flavonoids and its derivates, are secondary plant metabolites known for their antioxidant properties, that serve as radical scavengers and UV-absorbing compounds, shielding plant cells from radiation (Simontacchi et al., 2015). According to the literature data, the biosynthesis of flavonoids related to different solar radiation conditions depends on specific transcription factors, as R2R3 MYB and could be affected by the fluctuation of temperature (Neugart et al., 2016; Azuma et al., 2012). Carotenoids are bioactive compounds that protect chlorophyll from exposure to high light intensity.

This study is focused on thoroughly exploring how the reduction of solar radiation significantly impacts the morphometric characteristics and biochemical profiles of two distinct genotypes of commercial rocket plants, *Eruca sativa* L. var. *darkita* and var. *lobata*, and one Sicilian Black sprouting broccoli (*Brassica oleracea* L. var. *italica* Plenck, Broccolo nero) at different growth stages, including sprouts, microgreens, and baby-leaves. Sicilian black broccoli, a neglected landrace grown on the west slopes of Mount Etna in Sicily, shows distinctive plant morphology and a specific biochemical profile. This landrace is characterized by the absence of apical dominance, which determine the branching habitus of the plant which produce at the same time several small inflorescences. Moreover, the stems, leaves, and inflorescences are rich in anthocyanins, which provide them with a deep violet coloration. Several studies have demonstrated its high antioxidant capacity and glucosinolates profile, particularly for glucoraphanin content (Terzo et al., 2018, 2020). The plants were evaluated for their principal morphometric parameters and their biochemical phenotyping in relation to the experimental factors considered. The total phenolic content (TPC), the total flavonoid content (TFC), various antioxidant assays, such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ORAC (oxygen radical absorbance capacity), and FRAP (ferric reducing antioxidant power), were determined. Additionally, the total glucosinolates content and the sugar analysis were performed. The results allow the identification of genotypes that can be utilized under low solar radiation conditions.

2. Materials and methods

2.1. Experimental design

The seeds of the Sicilian landrace, Broccolo nero (BN, *Brassica oleracea* var. *italica* Plenck) belong to the Di3A active Brassica collection and the seeds of two genotypes of rocket (RU1 and RU2, *Eruca sativa* L. var. *darkita* and var. *lobata*, respectively) were provided by the S.A.I.S. S.p.A. seed company (Cesena, Italy). The seeds were planted on September 1st, 2022, in Catania, located in South Italy (37°31′10″ N, 15°04′18″ E; elevation 105 m above sea level), within cellular trays positioned in a cold greenhouse. The cultivation adhered to organic growing practices, maintaining natural light and temperature conditions averaging 30.73 ± 3.08 °C, alongside an average relative humidity of 62 %. The cellular trays were filled utilizing the organic substrate Brill®semina bio (Agrochimica S.p.A., Bolzano, Italy) and the plantlets were irrigated following the ordinary techniques. The randomized split-plot experimental design was adopted with three experimental factors: the first was represented by solar radiation (SR) adopting SR100 for 100 % solar radiation, SR60 for reduction of 40 % of solar radiation, and SR40 for the reduction of 60 % of the solar radiation, the second one was represented

by the different growth stages of the plant (GS), such as sprouts, microgreens and baby-leaves, and the third was the genotype (GE), utilizing BN, RU1 and RU2. Each experimental unit was articulated by three replicates of 1000 plants for sprouts, 200 plants for microgreens, and 100 plants for baby-leaves, for each of them. Two different black polyethylene shade nets were utilized (Arrigoni, Agrotextiles & Techtextiles, Como, Italy): 3050NE SCIROCCO 50 (hole dimension 0,97 × 1,39 mm) and 3070NE SCIROCCO 75 (hole dimension 0,35 × 1,39 mm), indicated as SR60 and SR40, respectively. The control, without net, was indicated as SR100. The light intensity was measured by LI-COR Quantum/Radiometer/Photometer (Model LI-250 Light Meter) every week from the beginning to the end of the trial. For the climatic conditions, significant variations were observed (Fig. 1, Figure S1). The solar radiation reduction was about 60 % from SR100 to SR40. One potential limitation of the experimental design is the limited number of genotypes studied. This study represents the starting point for identifying genotypes that are more efficient in relation to the reduction of solar radiation.

2.2. Morphometric characterization

The plants were collected at different growth stages: sprouts were represented by germinated seeds with just cotyledons, and they were collected after 7 days from sowing. Microgreens were harvested after 15 days after sowing, showing in addition of the cotyledons the first true leaf, whereas the baby-leaves were collected 1 month after sowing, showing about three-four true leaves. The plants were washed and characterized for their main morphometric traits: the weight of 10 individuals (W), hypocotyl length (HL), cotyledon length and width (CL and CW, respectively), for sprouts; in addition, number (N), length and width of the true leaves (LL and LW, respectively) for microgreens. Additionally, the stem length (SL) was determined for baby-leaves. The morphometric measurements were performed using Epson Perfection V850 Pro (WinFolia Regular 2020, https://regent.qc.ca/FR_root/assets/winfolia_about.html). After characterization, the plants were frozen at −80 °C, freeze-dried, and ground into a fine powder, which was then used for biochemical analysis.

2.3. Phytochemical measurements

The extracts for phytochemical measurement were obtained by homogenizing 50 mg of freeze-dried material with 1 mL of aqueous methanol (80/20, v/v) using 2.4 mm metal beads (Omni kit 19–670, Kennesaw, GA, USA) at room temperature for 1 min at 5 m/s (Omni-BeadRuptorElite, Kennesaw, GA, USA). The mixture, subsequently, was centrifuged at 16,000 x g for 10 min (Centric 350, Tehtnica). The supernatants were transferred to a clean tube and stored at −80 °C until further analysis.

2.3.1. Total phenolic content (TPC)

The total phenolic content (TPC) was measured following the Folin–Ciocalteu method, described by Singleton et al. (1999), with some modifications. 100 μL of the plant extract was mixed with 100 μL of 0.2 M (10 % v/v) Folin–Ciocalteu reagent and after one minute, 100 μL of a 6 % (w/v) sodium carbonate (Na_2CO_3) solution was added to the mixture. The mixture was incubated for 60 min at 25 °C, and the absorbance was measured at 750 nm using a Tecan Infinite 200 Pro M Nano+ spectrophotometer (Männedorf, Switzerland). The total phenolic content was obtained by a standard curve of gallic acid ($y = 3.995x - 0.0642$; serial dilutions of gallic acid: 20, 40, 60, 80, and 100 μg/mL; coefficient of determination: $R^2 = 0.9998$). The results were expressed as milligrams of Gallic Acid Equivalent per 100 g of dry weight (mg GAE/100 g d.w.).

2.3.2. Total antioxidant capacity measurement

2.3.2.1. Ferric reducing antioxidant power (FRAP). The FRAP assay was performed by mixing 100 μL of the sample extract with 200 μL of FRAP reagent according to the method of Benzie and Strain (1996). After a 10 min incubation at room temperature, the absorbance was measured at 593 nm using a Tecan Infinite 200 Pro M Nano+ spectrophotometer (Männedorf, Switzerland). The FRAP values were determined based on a Trolox+ calibration curve ($y = 10.0044x - 0.094$; serial dilutions of Trolox: 20, 40, 60, 80, 100 μM; coefficient of determination: $R^2 = 0.9995$). The results were expressed as μmol of Trolox equivalents (TE) per gram of dry weight (d.w.).

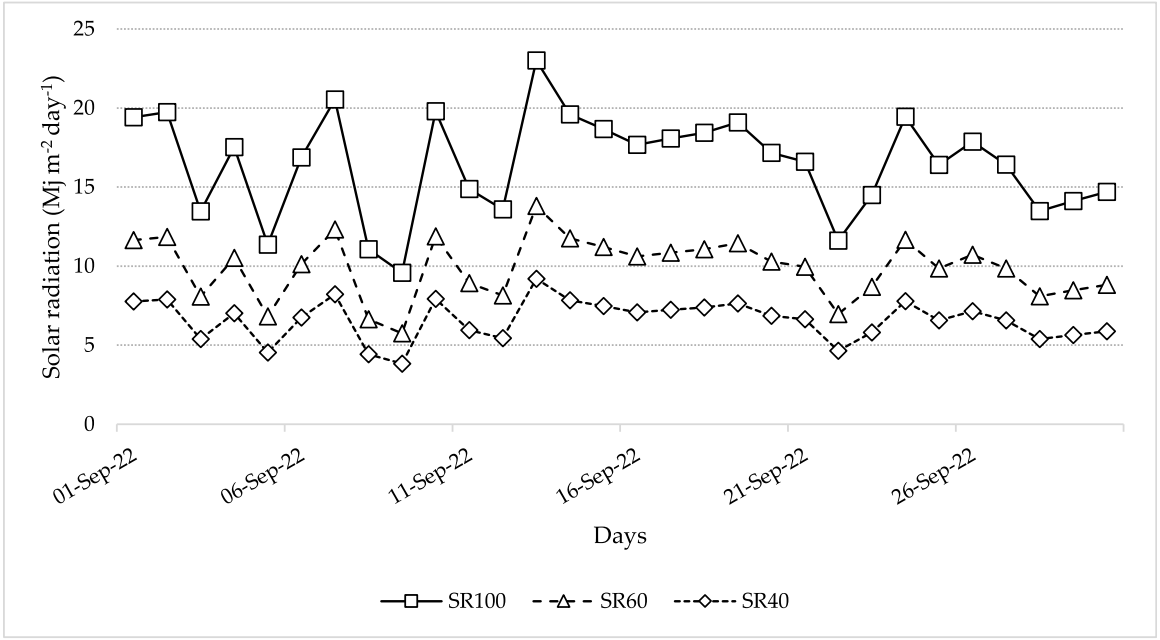

Fig. 1. Solar radiation for the experimental conditions tested (MJ m^{-2} day^{-1}).

2.3.2.2. 1,1-diphenyl-2-picrylhydrazil scavenging (DPPH). Following the methodology described by Brand-Williams et al. (1995), 100 μL of the sample extract was mixed with 200 μL solution of 0.1 mM DPPH reagent. Following a 30-minute incubation at room temperature, spectrophotometric analysis was conducted by measuring absorbance at 517 nm. These determinations were made using a Trolox+ standard curve ($y = -15.147x + 15.186$; serial dilutions: 20, 40, 60, 80, 100 μM; coefficient of determination, $R^2 = 0.9999$). The results were expressed as μmol TE per gram of dry weight (d.w.).

2.3.2.3. Oxygen radical absorbance capacity (ORAC). A total of 37.5 μL of the plant extract was combined with 225 μL of a 4 μM fluorescein solution and subjected to incubation at 37 °C for 30 min, following the protocol outlined by Ou et al. (2013). Subsequently, 37.5 μL of an AAPH solution was introduced into the mixture. Fluorescence measurements were conducted using a Tecan Infinite 200 Pro M Nano+ spectrophotometer (Männedorf, Switzerland), with excitation and emission filters set at 485 nm and 528 nm, respectively. Fluorescence readings were recorded at one-minute intervals over a 35-minute duration. The establishment of a standard curve was achieved by plotting Trolox concentrations against the average net Area Under the Curve (AUC) for each concentration. The determination of ORAC values were determined utilizing a Trolox+ standard curve against the AUC, net area under the curve ($y = 0.034x - 0.000249$), with serial dilutions of Trolox (8, 16, 24, 32, 40 μM). The coefficient of determination was $R^2 = 0.9998$. The resultant ORAC values were expressed as μmol Trolox equivalents per gram of dry weight (d.w.). The calculation of AUC was determined using the following formula:

$$AUC = 0.5 + f1/f0 + ...fi/f0 + \ldots + f34/f0 + 0.5 \times (f35/f0)$$

where f0 = initial fluorescence reading at 0 min and fi= fluorescence reading at time I.

The net AUC was obtained by subtracting the AUC of the blank from each sample.

2.3.2.4. Total flavonoid content (TFC). The Total Flavonoid Content was carried out using the aluminum chloride colorimetric assay method as described by Sembiring et al. (2018), with some adaptations. 100 μL of plant extract was combined with 10 μL of a 10 % aluminum chloride solution. 180 μL of 80 % methanol and 10 μL of 1 M sodium acetate were added. The mixture was incubated for 40 min at room temperature in dark conditions. The absorbance was recorded at 415 nm using a Tecan Infinite 200 Pro M Nano+ spectrophotometer (Männedorf, Switzerland). The results were obtained by a standard curve of Quercetin ($y = 7.1021x - 0.005$; serial dilutions of Quercetin ranging from 20 to 100 mg/L; coefficient of determination: $R^2 = 0.9999$). The TFC values were expressed in terms of milligrams of Quercetin (QE) per gram of dry weight (d.w.).

2.3.2.5. Total glucosinolates content (SEQ). The total glucosinolates content was measured according to Ishida et al. (2012), with some modifications.100 μL of the plant extract was combined with 100 μL of 0.2 M Na_2PdCl_4. After one minute, 100 μL of 6 % (w/v) sodium carbonate (Na_2CO_3) solution was added. The mixture was then incubated at 25 °C for 60 min, following absorbance measurement at 425 nm using a Tecan Infinite 200 Pro M Nano+ spectrophotometer (Männedorf, Switzerland). The quantification was carried out using a standard curve of sinigrin ($y = 3.7585x - 0.0035$), involving serial dilutions ranging from 20 to 100 μg/mL. This calibration exhibited a high coefficient of determination ($R^2 = 0.9997$). The results were expressed in terms of gram of Sinigrin Equivalents (SEQ) per gram of dry weight (d.w.).

2.4. Soluble sugar analysis

An amount of 150 mg of the freeze-dried samples was homogenized with 2.4 mm metal beads (Omni kit 19–670, Kennesaw, GA, USA) for 1 min at 5 m s^{-1} in 3 mL of 80 % methanol in water using a bead mill (Omni Bead Ruptor Elite, Kennesaw, GA, USA). The samples were subsequently centrifuged for 5 min at 5000 × *g*. The extracts were filtered through a 0.22 μm nylon filter. The analysis of sucrose, glucose, fructose, and inulin (FOS) content was carried out using an HPLC system with a system controller (Shimadzu CBM-40, Kyoto, Japan), a degassing unit (Shimadzu DGU-405, Kyoto, Japan), a solvent delivery unit (Shimadzu LC-20Ai, Kyoto, Japan), an autosampler (Shimadzu SIL-20AC, Kyoto, Japan), column oven (Shimadzu CTO-40S, Kyoto, Japan) and a refractive index detector (Shimadzu RID-20A, Kyoto, Japan). The chromatographic separation was carried out by injecting 10 μL of the sample on a 300 × 8 mm, 9 μm particle size, calcium cation exchange column (Ammerbuch, Germany) held at 80 °C using deionized water as the mobile phase (0.6 mL min^{-1}, isocratic elution). The results were elaborated by comparing the retention times and peak areas of the sugars to analytical standards for identification and quantification, respectively. The calibration curves were obtained with serial dilutions of inulin (0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 g L^{-1}) ($y = 114.85x - 1.743$, coefficient of determination, $R^2 = 0.99999$), sucrose ($y = 130.79x - 0.3583$, coefficient of determination, $R^2 = 0.99999$), glucose ($y = 132.36x - 2.101$, coefficient of determination, $R^2 = 0.9999$) and fructose ($y = 126.99x - 1.4761$, coefficient of determination, $R^2 = 0.9999$). Total sugars are the sum of extractable sugars, such as FOS, sucrose, glucose, and fructose. The results were given in grams/1000 gs of dry weight (d.w.). According to literature data, soluble sugars such as glucose, fructose, and sucrose are involved in the growth and development of plants, including cell division and differentiation, photosynthesis, carbohydrate and lipid metabolism, and responses to abiotic stressors such as temperature, drought stress, and light (Arbona et al., 2013; Halford et al., 2011). Each sugar plays a different role in response to stress, as sucrose and glucose maintain cell homeostasis, while fructose is involved in the synthesis of metabolites (Rosa et al., 2009). Several studies have shown that under environmental stress conditions, the soluble sugars content varies significantly, also depending on the crop variety and the specific stress (Fareen et al., 2016). The variation of solar radiation can affect sugar metabolism and, consequently, crop productivity, yield, and plant development. Numerous studies have shown the accumulation of various carbohydrates, such as fructose, sucrose, and FOS in response to abiotic stresses (Hasperué, et al., 2011; Rosales et al., 2007).

2.5. Statistical analysis

The analysis of variance (ANOVA) was performed to evaluate the differences among the experimental factors considered, using CoStat version 6.451 (CoHort Software, Birmingham, England). A two-way ANOVA was adopted to evaluate the effects of solar radiation and genotype on morphometric plant traits. The significance of the differences for the biochemical traits were elaborated by three-way ANOVA. Tukey's post-hoc test ($p < 0.05$) was performed using CoStat version 6.451 (CoHort Software, Birmingham, England) to determine the significant differences in relation to the experimental factors studied. The graphical representation of the climatic conditions was generated using R Studio version 3.6.3., to determine the minimum reduction in solar radiation required to select the most efficient genotype. Pearson's correlation coefficient was performed using the SPSS software version 27 to evaluate the relationship between the TPC, antioxidant activity, TFC, total glucosinolates and sugars. The Principal Component Analysis (PCA) was conducted using the XLSTAT2018 software (Addinsoft, Paris, France).

3. Results

3.1. Morphometric characterization

The morphometric parameters of the sprouts displayed variations dependent on two key experimental factors, solar radiation (SR) and genotype (GE), as summarized in Table 1. Notably, a significant interaction was observed for the weight, for which the values ranged from 8.30 ± 0.10 g to 2.56 ± 0.04 g, for the BN SR60 and RU1 SR100, respectively.

The hypocotyl length was affected by genotype, and its value varied from 19.88 ± 1.24 mm to 25.27 ± 2.29 mm for RU1 and BN, respectively. The cotyledon length did not exhibit any significant differences among the experimental factors considered. However, cotyledon width was notably affected by SR, ranging from 11.89 ± 0.66 mm to 9.85 ± 0.87 mm in SR100 and SR40, respectively. These results highlight the complex influence of both solar radiation (SR) and genotype (GE) on the morphometric characteristics of the sprouts, suggesting potential implications for plant growth and development. RU2 sprouts showed the highest values of weight and hypocotyl length considering the 60 % of solar reduction (SR40) compared to the other genotypes.

For microgreens, we ascertained a significant interaction between SR and GE for hypocotyl length, and its values varied from 50.80 ± 0.54 mm to 19.90 ± 0.15 mm, for BN SR60 and RU2 SR100, respectively (Table 2). No significant differences were observed for the weight. The cotyledon length was significantly affected by the genotype, with values ranged from 10.52 ± 0.07 mm to 8.95 ± 0.03 mm, for BN and RU1, respectively. A significant interaction was observed for cotyledon width, whose value varied from 10.08 ± 0.08 to 9.72 ± 0.05 mm, for BN SR60 and SR40, respectively. The value was higher compared to RU1 SR60 and SR40 (7.92 ± 0.03 and 7.97 ± 0.04 mm, respectively) and RU2 SR60 and SR40 (8.23 ± 0.02 and 8.96 ± 0.07 mm, respectively). No significant variations were ascertained for leaf length. The highest value was ascertained for BN SR100 (38.15 ± 0.02 mm), whereas the lowest one was determined for RU2 SR40 (21.20 ± 0.04 mm). BN was the most performant genotype until low solar radiation.

For the baby-leaves, no significant variations were ascertained for the weight (Table 3). The stem length was affected by the solar radiation (SR), with values ranging from 64.85 ± 0.43 mm to 22.37 ± 0.19 mm, at SR40 and SR100, respectively. The stem length was affected also by the genotype, and BN showed the highest values (66.73 ± 1.92 mm), while the lowest one was ascertained for RU2 (35.20 ± 1.88 mm). The number of leaves was significantly affected by the genotype and the value ranged from 3.82 ± 0.12 to 3.25 ± 0.11, for RU2 and BN, respectively. Notably, a significant interaction between SR and GE was ascertained for leaf length, with values varying from 43.59 ± 0.27 to 26.83 ± 0.30 mm, for RU1 SR60 and BN SR100, respectively. Furthermore, leaf width was notably affected by the genotype, with BN shown the highest value (25.50 ± 0.17 mm). These results show the relation between genetic factors and environmental conditions in shaping the morphometric traits of baby-leaves. RU2 SR40 in general, exhibited the highest values during this plant growth stage.

Table 1
Morphometric characteristics of the sprouts of the Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2) studied. Data are reported as mean ± *S*.E. ($n = 10$). *W* = weight of 10 individuals (g); HL = hypocotyl length of 10 individuals (mm); CL = cotyledon length of 10 individuals (mm); CW = cotyledon width of 10 individuals (mm).

		W (g)	HL (mm)	CL (mm)	CW (mm)
Solar radiation (SR)					
	SR100	4.27 ± 0.26	20.89 ± 2.55	10.65 ± 0.42	11.89 ± 0.66 a
	SR60	4.93 ± 0.30	24.16 ± 3.74	9.84 ± 0.38	11.17 ± 1.63 ab
	SR40	3.77 ± 0.06	23.9 ± 2.93	9.39 ± 0.60	9.85 ± 0.87 b
		Ns	ns	ns	*
Genotype (GE)					
	BN	6.30 ± 0.26 a	25.27 ± 2.29 a	9.65 ± 0.91	10.33 ± 1.36
	RU1	3.37 ± 0.07 b	19.88 ± 1.24 b	9.99 ± 0.48	12.14 ± 1.22
	RU2	3.30 ± 0.10 b	23.80 ± 2.99 a	10.25 ± 0.75	10.44 ± 0.80
		***	**	ns	ns
SR × GE					
SR100	BN	7.33 ± 0.11 ab	23.56 ± 5.17	10.59 ± 2.54	11.72 ± 4.26
	RU1	2.56 ± 0.04 c	18.47 ± 2.60	10.27 ± 0.59	12.62 ± 0.93
	RU2	2.90 ± 0.05 c	20.63 ± 4.74	11.12 ± 0.58	11.33 ± 0.69
SR60	BN	8.30 ± 0.10 a	27.87 ± 1.79	9.58 ± 1.65	10.27 ± 0.68
	RU1	3.87 ± 0.19 bc	20.40 ± 0.85	10.27 ± 0.38	13.05 ± 0.95
	RU2	2.63 ± 0.04 c	24.20 ± 4.81	9.67 ± 0.88	10.18 ± 0.55
SR40	BN	3.27 ± 0.18 bc	24.37 ± 1.10	8.77 ± 1.01	9.01 ± 1.54
	RU1	3.60 ± 0.10 bc	20.77 ± 1.51	9.43 ± 0.60	10.75 ± 1.10
	RU2	4.36 ± 0.25 bc	26.57 ± 2.71	9.97 ± 0.32	9.80 ± 0.35
		**	ns	ns	ns

The mean values associated with the two factors and their interaction were evaluated according to Tukey's test. Means significantly different are indicated by different letters. n.s. not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.2. Phytochemical measurements

The results of the ANOVA analysis reveal important insights about the factors influencing various parameters in the study (Table 4). Genotype emerges as the experimental factor determinant, significantly impacting antioxidant properties (DPPH, FRAP), total flavonoid content (TFC), and other compounds (TGLSs, glucose) of the novel foods proposed. Meanwhile, the plant growth stage (GS) indicates the dynamic changes in antioxidant properties, flavonoid content, and sugars detected during the first stages of the plant growth. Interestingly, solar radiation primarily affects the sugar composition, suggesting its role in modulating plant sugar amount into the products suggested.

3.2.1. Total phenolic content (TPC)

TPC is a critical parameter as phenolic compounds are well-known for their antioxidant properties. No significant variations in response to solar radiation (SR) and genotype (GE) were detected. However, the TPC value was significantly affected by the plant growth stage (GS) (Table 4). The highest value was registered for baby-leaves (8.74 ± 0.23 mg GAE/100 g d.w.), closely followed by sprouts and microgreens (7.96 ± 0.18 and 7.46 ± 0.11 mg GAE/100 g d.w., respectively). Notably, we observed an increase in TPC content from sprouts to baby-leaves, meaning an accumulation of the phenolic compounds with the growth of the plants (Table 4). The tested genotypes are a good source of total phenolic content, in particular during the baby-leaves stage.

3.2.2. Total antioxidant capacity

The results of the FRAP activity analysis, detailed in Table 4, reveal interesting insights. While solar radiation (SR) variations did not significantly impact the results. The antioxidant capacity displayed notable variations that were markedly influenced by the genotype. In this regard, the BN genotype emerged as the top performer, boasting an impressive average of 39.97 μmol TE/g dry weight. BN significantly showed high values in comparison to RU1 (19.18 μmol TE/g d.w.) and RU2 (22.64 μmol TE/g d.w.).

Regarding the DPPH activity, the influence of genotype is highly

Table 2
Morphometric characteristics of the microgreens of the Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2) studied. Data are reported as mean ± *S*.E. ($n = 10$). *W* = weight of 10 individuals (g); HL = hypocotyl length of 10 individuals (mm); CL = cotyledon length of 10 individuals (mm); CW = cotyledon width of 10 individuals (mm); LL = leaf length of 10 individuals (mm); LW = leaf width of 10 individuals (mm).

		W (g)	HL (mm)	CL (mm)	CW (mm)	LL (mm)	LW (mm)
Solar radiation (SR)							
	SR100	5.50 ± 0.12	22.13 ± 0.32 b	9.44 ± 0.03	8.50 ± 0.03	45.04 ± 0.10	29.04 ± 0.08 a
	SR60	5.47 ± 0.04	35.03 ± 1.38 a	9.70 ± 0.12	8.73 ± 0.12	36.11 ± 0.02	24.10 ± 0.02 b
	SR40	5.63 ± 0.05	32.47 ± 0.66 a	9.81 ± 0.11	8.90 ± 0.09	36.72 ± 0.02	23.22 ± 0.02 b
		ns	***	ns	ns	ns	**
Genotype (GE)							
	BN	5.07 ± 0.09	38.70 ± 1.25 a	10.52 ± 0.07 a	9.57 ± 0.06 a	38.73 ± 0.07	29.08 ± 0.08 a
	RU1	5.70 ± 0.03	27.27 ± 0.58 b	8.95 ± 0.03 b	8.07 ± 0.02 b	42.71 ± 0.10	25.15 ± 0.02 b
	RU2	5.83 ± 0.07	23.67 ± 0.34 b	9.56 ± 0.05 b	8.50 ± 0.04 b	36.30 ± 0.03	22.11 ± 0.01 b
		ns	***	***	***	ns	**
SR × GE							
SR100	BN	4.24 ± 0.20	25.80 ± 0.39 c	9.81 ± 0.86	8.87 ± 0.05 ab	46.51 ± 0.01	38.15 ± 0.02 a
	RU1	5.77 ± 0.12	20.70 ± 0.16 c	9.32 ± 0.45	8.33 ± 0.04 b	53.55 ± 0.19	27.34 ± 0.05 ab
	RU2	6.53 ± 0.23	19.90 ± 0.15 c	9.32 ± 0.36	8.27 ± 0.03 b	33.82 ± 0.07	22.05 ± 0.04 b
SR60	BN	5.93 ± 0.36	50.80 ± 0.54 a	11.13 ± 0.82	10.08 ± 0.08 a	34.43 ± 0.05	24.17 ± 0.03 b
	RU1	5.43 ± 0.10	29.62 ± 0.37 c	9.03 ± 0.63	7.92 ± 0.03 b	37.64 ± 0.07	25.21 ± 0.03 b
	RU2	5.15 ± 0.14	24.67 ± 0.29 c	9.15 ± 0.19	8.23 ± 0.02 b	35.50 ± 0.03	22.16 ± 0.02 b
SR40	BN	5.13 ± 0.27	39.53 ± 0.88 ab	10.72 ± 0.81	9.72 ± 0.05 a	34.61 ± 0.08	25.01 ± 0.07 b
	RU1	5.93 ± 0.01	31.50 ± 0.25 bc	8.65 ± 0.18	7.97 ± 0.04 b	35.67 ± 0.03	23.11 ± 0.02 b
	RU2	5.90 ± 0.04	26.40 ± 0.28 c	10.16 ± 0.63	8.96 ± 0.07 ab	38.91 ± 0.04	21.20 ± 0.04 b
		ns	**	ns	*	ns	*

The mean values associated with the two factors and their interaction were evaluated according to Tukey's test. Means significantly different are indicated by different letters. n.s. not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

significant (19.50 ± 2.37 μmol TE/g d.w.). This value is more than doubled in comparison to RU1 and RU2, which recorded 7.81 ± 1.66 and 8.68 ± 2.44 μmol TE/g dry weight, respectively.

The interaction between the three factors, solar radiation, growth stage and genotype, had a highly significant impact on the ORAC levels (p-value= 0.0001***), as depicted in Table 4. Notably, baby-leaves of the BN genotype cultivated in SR40 exhibited the highest ORAC values (229.79 μmol TE/g d.w.), as illustrated in Fig. 2. This suggests that they present a remarkable antioxidant potential. RU1 sprouts showed the lowest ORAC values grown by SR100 (137.81 μmol TE/g d.w.) (Table 4). The antioxidant capacity measured by different methods varied significantly in relation to the genotypes, particularly for ORAC assay a significant interaction between SR, GS and GE was observed.

3.2.3. Total flavonoid content (TFC)

Significant variations in the Total Flavonoid Content (TFC) were observed based on the genotype (Fig. 3). RU2 exhibited the highest TFC value (5.41 ± 1.25 mg QE/g d.w.), closely followed by RU1 (4.70 ± 1.41 mg QE/g d.w.). RU2 microgreens grown in SR40, showed the highest value (7.89 ± 0.73 mg QE/g d.w.). Interestingly, TFC tended to increase during the plant growth from the sprout stage to the baby-leaves, with the highest values observed for microgreens and baby-leaves plantlets. Specifically, for baby-leaves, TFC value ranged from 6.81 ± 1.22 to 4.75 ± 0.76 mg QE/g d.w. (Table 4). RU2 showed the highest total flavonoid content increasing from sprouts to baby-leaves. The TFC was highest under the 60 % solar radiation reduction.

3.2.4. Total glucosinolates content (SEQ)

The total glucosinolate content shows a significant interaction between the genotype and plant growth stage and the values ranged from 6.96 ± 0.66 g SEQ/g d.w. for the BN genotype to 5.56 ± 0.89 g SEQ/g d.w. for the RU2 genotype (Fig. 4). Remarkably, RU1 showed a comparable value with RU2, with an amount of 5.57 ± 1.20 g SEQ/g d.w. The overall profile of the glucosinolates content exhibited similarities among the different growth stages, generally showing a tendency to increase from sprouts to baby-leaves for RU1 and RU2, while for BN the highest amount was detected in the microgreens stage. The total glucosinolates content varied significantly in relation to the genotypes studied, and in particular BN exhibited the highest amount.

3.2.5. Soluble sugar analysis

The analysis of total sugar content reveals a significant interaction SR × GS × GE. For the sprout growth stage, BN exhibited the highest total sugar content in control condition, which notably decreased as solar radiation were reduced, and its lowest value was observed for SR60 and then recovering slightly for SR40. Conversely, genotype RU1 had a relatively stable total sugar content across all SR levels indagated, with only minor fluctuations. RU2 followed a similar pattern observed for BN, starting with higher sugar content by SR100, decreasing by SR60, and then recovering by SR40. The total sugar content for the sprouts stage, was higher for BN, indicating its better sugar accumulation capability, followed by RU2 and RU1 (Fig. 5).

For the microgreen's growth stage, BN showed once again the highest sugar content at SR100, which decreased by decreasing the SR. RU1, however, experienced a decline by SR60 before rebounding by SR40. RU2 displayed a relatively stable sugar content throughout all solar radiation levels tested. During the microgreens stage, BN exhibited the highest total sugar content, followed by RU2 and RU1 in descending order of sugar accumulation.

For the baby-leaves growth stage, BN showed relatively consistent total sugar content among the different solar radiation levels, indicating its resilience to light variation. RU1 showed some variations but generally maintained stability for sugar content. Conversely, RU2 exhibited a noticeable drop in sugar content as solar radiation decreased. Through a detailed examination of the total sugar content for the baby-leaves growth stage, RU2 displayed the most elevated values, and BN and RU1 closely trailing behind in the hierarchy of sugar accumulation. Considering the total sugar content during the three growth stages studied and genotypes, RU2 showed the highest capacity for sugar accumulation, indicating its resilience to solar radiation reduction. For BN, the value was high for the sprouts and microgreens stages, but still had a relatively high overall mean. RU1 displayed the lowest value, indicating its lower ability to accumulate sugars under solar radiation reduction. This analysis highlights how different genotypes respond to solar radiation variation during different growth stages in terms of total sugar content. RU2 appears the best one in sugar accumulation, while BN shows its strength in the early stages of growth. Finally, RU1, on the other hand, exhibits some vulnerability to solar radiation reduction, resulting in lower sugar content. These findings

Table 3
Morphometric characteristics of the baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2) studied. Data are reported as mean ± *S*.E. ($n = 10$). *W* = weight of 10 individuals (g); SL = stem length of 10 individuals (mm); *N* = number of true leaves of 10 individuals (n); LL = leaf length of 10 individuals (mm); LW = leaf width of 10 individuals (mm).

		W (g)	SL (mm)	N (n)	LL (mm)	LW (mm)
Solar radiation (SR)						
	SR100	3.04 ± 0.50	22.37 ± 0.19 b	3.73 ± 0.35	32.27 ± 0.55 b	18.20 ± 0.47
	SR60	4.56 ± 1.40	57.26 ± 0.81 a	3.55 ± 0.35	37.33 ± 0.55 a	19.17 ± 0.65
	SR40	4.17 ± 0.60	64.85 ± 0.43 a	3.48 ± 0.31	35.13 ± 0.51 ab	20.97 ± 0.47
		ns	***	ns	*	ns
Genotype (GE)						
	BN	4.01 ± 1.83	66.73 ± 1.92 a	3.25 ± 0.11 b	30.90 ± 0.41 b	25.50 ± 0.17
	RU1	4.00 ± 0.58	48.90 ± 2.03 b	3.78 ± 0.20 ab	38.30 ± 0.50 a	15.97 ± 0.22
	RU2	3.78 ± 0.77	35.20 ± 1.88 b	3.82 ± 0.12 a	35.53 ± 0.46 ab	16.87 ± 0.11
		ns	***	***	**	***
SR × GE						
SR100	BN	2.47 ± 1.86	44.63 ± 0.54	3.33 ± 0.15	26.83 ± 0.30 c	23.50 ± 0.33
	RU1	3.33 ± 1.53	26.81 ± 0.59	3.97 ± 0.06	37.73 ± 0.1 abc	14.38 ± 0.24
	RU2	3.33 ± 1.15	14.71 ± 0.13	3.92 ± 0.17	32.33 ± 0.35 abc	16.70 ± 0.19
SR60	BN	6.02 ± 1.0	79.20 ± 2.58	3.30 ± 0.10	34.94 ± 0.15 abc	26.55 ± 0.54
	RU1	4.33 ± 0.58	53.31 ± 0.39	3.80 ± 0.20	43.59 ± 0.27 a	15.01 ± 0.17
	RU2	3.33 ± 0.58	39.28 ± 0.30	4.01 ± 0.00	33.47 ± 0.62 abc	15.87 ± 0.21
SR40	BN	3.50 ± 2.78	76.37 ± 2.40	3.13 ± 0.23	31.11 ± 0.13 bc	26.37 ± 0.07
	RU1	4.33 ± 0.58	66.57 ± 1.02	3.57 ± 0.38	33.57 ± 0.55 abc	18.53 ± 0.31
	RU2	4.67 ± 1.53	51.63 ± 0.72	3.73 ± 0.38	40.79 ± 0.31 ab	17.94 ± 0.06
		ns	Ns	ns	*	ns

The mean values associated with the two factors and their interaction were evaluated according to Tukey's test. Means significantly different are indicated by different letters. n.s. not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

provide valuable insights into the sugar dynamics of the studied genotypes under different solar radiation conditions.

The glucose content (g/1000 g d.w.) varied significantly in relation to the solar radiation showing a significant interaction GS × GE. The glucose amount varied in relation to solar radiation from 11.83 to 21.51 g/1000 g d.w. for SR60 and SR40, respectively (Table 4). The interaction between GS × GE affected the glucose content which varied from 4.50 to 30.59 g/1000 g d.w., for BN sprouts and RU2 microgreens, respectively (Fig. 6). The glucose amount increases generally from sprouts to baby-leaves for all the genotypes evaluated.

The fructose amount varied significantly in relation to the solar radiation which varied from 5.73 to 11.24 for SR60 and SR40, respectively (Table 4). The interaction between GS × GE affected the fructose content ranging from 3.72 to 15.24 g/1000 g d.w., for BN microgreens and RU1 sprouts, respectively (Fig. 7). The fructose amount increases from sprouts to baby-leaves for BN, while decreasing during the growth of the plants, for RU1 and RU2.

The sucrose amount varied significantly in relation to the solar radiation showing, as mentioned before for glucose, a significant interaction GS × GE. The sucrose content varied, in relation to solar radiation between 7.96 to 11.47 g/1000 g d.w., for SR100 and SR40, respectively (Table 4). The interaction between GS × GE affected the sucrose content ranging from 2.29 to 15.43 g/1000 g d.w. for RU2 sprouts and baby-leaves, respectively (Fig. 8). The sucrose amount, as for glucose, increased from sprouts to baby-leaves for all the genotypes tested.

The FOS amount was significantly affected by the interaction SR × GS × GE (Table 4). The FOS values varied from 26.93 to 216.28 g/1000 g d.w., for RU1 microgreens SR60 to the BN microgreens SR100, respectively (Fig. 9). The FOS content, generally, decreased from sprouts to baby-leaves. For total sugars and FOS, significant interactions SR × GS × GE were observed. The soluble sugars content was affected by the genotype and an increase in sugar content was ascertained with the 60 % of solar radiation reduction.

3.3. Correlation and principal component analysis

Exploring the effects of varying the solar radiation on plant physiological parameters, particularly TPC, antioxidant capacity and sugar component, the results reveal significant correlations that provide insight into the complex biochemical dynamics of plant responses (Table 5). Notably, the antioxidant capacity, assessed through TPC, DPPH, FRAP and ORAC assays, demonstrated positive correlations (DPPH-FRAP: $r = 0.921$**, TPC—ORAC: $r = 0.336$**, FRAP-ORAC: $r = 0.377$**) indicative of a synchronized response to solar radiation variation. TFC is positively correlated with glucose and ORAC ($r = 0.549$** and $r = 0.249$*, respectively) emphasizing the influence of these secondary metabolites. The glucose showed a negative correlation with DPPH ($r = -0.322$**), FRAP ($r = -0.274$*) and TGLSs ($r = -0.221$*), suggesting a potential regulatory role of glucose in modulating antioxidant response. Furthermore, sucrose displayed a high positive correlation with the glucose ($r = 0.817$**) underscoring the intricate relationship within the sugar profile. The fructooligosaccharides (FOS) are highly correlated with the total sugar ($r = 0.953$**) highlighting its central role in sugar composition.

In the analysis of three distinct growth stages (sprouts, microgreens, and baby-leaves), the correlation coefficients reveal intriguing insights into the relationships among various phytochemical properties. The correlation matrix provides a comprehensive overview of the associations between TPC, antioxidant activities (DPPH, FRAP, ORAC), TFC, TGLSs, and sugar profiles (glucose, fructose, sucrose, and FOS) in each stage.

The heatmaps of the Pearson correlation between the different biochemical compounds detected are presented in supplementary tables. Whereby SR100, the TPC demonstrates moderate to strong positive correlations with various antioxidant capacity measurements, including, FRAP ($r = 0.456$*), TGLSs ($r = 0.475$*) and ORAC ($r = 0.648$**), indicating that as TPC levels rise, so do these antioxidant capacities (Table S1). Additionally, DPPH and FRAP exhibit a notably strong positive correlation ($r = 0.932$**). In contrast, TFC displays high positive correlations with sucrose ($r = -0.718$**). Furthermore, sugar-related variables, including sucrose, glucose, fructose, and total sugars, demonstrate strong positive correlations among them, particularly sucrose, and glucose ($r = 0.815$**).

In SR60, TPC demonstrates substantial positive correlations with TFC (0.431) (Table S2). Secondly, a particularly strong positive correlation of 0.971 is observed between DPPH and FRAP. This high correlation coefficient suggests that DPPH and FRAP provide largely redundant information regarding antioxidant properties, indicating their interchangeability in assessing antioxidant capacity. Furthermore, the strong correlation of 0.883 between sucrose and glucose underscores a substantial association between these two sugar-related variables. Total sugars exhibit a strong positive correlation of 0.986 with FOS. This result suggests a robust and consistent association between total sugars and FOS, implying that changes in one variable are strongly mirrored by changes in the other. The analysis reveals strong correlations between TFC and sugar content, including glucose (0.609) and sucrose (0.566).

In SR40, the TPC shows strong positive associations with TGLSs

Table 4
Comparative analysis of antioxidant properties and sugar content of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2) considering different initial plant growth stage (GS), as sprouts (SP), microgreens (MG), baby-leaves (BL), and different solar radiation reduction (SR). TPC= Total Phenolic Content; FRAP= Ferric Reducing Antioxidant Power; DPPH= 1,1-diphenyl-2-picrylhydrazil scavenging; ORAC= Oxygen radical absorbance capacity; TFC= Total Flavonoid Content; TGLSs= Total glucosinolates content; FOS= fructooligosaccharides.

	TPC	FRAP	DPPH	ORAC	TFC	TGLSs	Total sugar	Glucose	Fructose	Sucrose	FOS
SR											
SR100	8.10 ±0.27	27.23 ±1.93	12.63 ±1.33	169.51 ±5.70	4.74 ±0.28b	6.14±0.25 ab	181.89±9.91	12.81 ±1.59 b	7.33±0.94 b	7.96±0.96 b	153.80 ±10.37 a
SR60	8.04 ±0.14	26.97 ±1.23	11.55 ±1.12	178.71 ±5.03	4.50 ±0.24b	6.26 ±0.28a	186.32± 15.83	11.83 ±1.77b	5.73±0.71 b	7.35±0.90 b	143.64± 14.62 b
SR40	8.02± 0.20	27.58 ±2.02	11.81± 1.11	179.19± 5.48	5.51±0.25 a	5.68±1.89 b	174.91±5.95	21.51 ±2.39 a	11.24 ±1.45 a	11.47 ±1.42 a	142.12±8.72 b
GS											
SP	7.96± 0.18 b	27.02 ± 2.10	12.80 ±1.31	166.20 ± 4.50 b	4.17 ± 0.21 b	5.72± 0.30	199.25 ±12.40 a	7.02± 1.1 b	10.52± 1.13 a	3.95± 0.53 c	186.48± 11.79 a
MG	7.46± 0.11 b	26.23 ±1.70	11.05 ±1.01	176.25 ±4.75 ab	4.85±0.32 b	6.18±0.26	195.31 ±14.31 a	17.84 ±2.36 a	4.70±0.42 b	8.81±0.96 b	148.86± 13.50 b
BL	8.74 ± 0.23 a	28.52 ±1.93	12.14 ±1.22	184.95± 6.36 a	5.73±0.17 a	6.19 ±0.145	148.56± 4.24 b	21.28 ±1.58 a	9.07± 1.40 a	14.01 ±0.99 a	104.21±4.51 c
GE											
BN	8.32 ±0.24	39.97± 0.86 a	19.49± 0.73 a	188.96± 5.73 a	4.63±0.24 b	6.96±0.29 a	178.45 ± 13.14 b	9.86±1.72 b	8.98±1.36	7.34±0.81 b	160.99 ±13.82 ab
RU 1	7.70± 0.14	19.18± 0.45 c	7.81±0.40 b	167.23 ±3.85 b	4.70±0.24 ab	5.57±0.11 b	167.43 ±12.45 b	15.981 ±1.6 a	8.313 ±1.25	8.82±1.23 ab	159.33 ±11.55 b
RU 2	8.13± 0.21	22.63 ±0.82 b	8.68± 0.59b	171.23± 5.57 b	5.4 ± 0.30 a	5.56± 0.90 b	197.24± 6.01 a	20.30± 2.44 a	7.00± 0.77	10.62± 1.33 a	119.23± 7.70 b
Significancy of the differences by ANOVA Newman-Keuls method											
SR	n.s.	n.s.	n.s.	n.s.	**	*	n.s.	***	***	***	*
GS	***	n.s.	n.s.	*	***	n.s.	*	***	***	***	***
GE	n.s.	***	***	**	**	***	**	***	n.s.	*	***
SR × GS	n.s.	n.s.	n.s.	n.s.	***	n.s.	***	n.s.	**	n.s.	***
SR × GE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	***
GS × GE	n.s.	n.s.	n.s.	n.s.	***	***	***	***	***	***	***
SR × GS × GE	n.s.	n.s.	n.s.	***	n.s.	n.s.	***	n.s.	n.s.	n.s.	***

The interaction of the factors *, **, and *** indicate that the Tukey test is significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively.

(0.588). Additionally, DPPH and FRAP exhibit a strong positive correlation (0.885), indicating their interchangeability in assessing antioxidant capacity. Sucrose and glucose display a substantial association (0.751). Total sugars and FOS share a strong positive correlation (0.903), indicating their close relationship (Table S3). The findings highlight the main positive correlation between different antioxidant assays, such as DPPH, ORAC, and FRAP, suggesting a high reactivity of ORAC with polyphenol compounds. Additionally, we observed that FOS are the main sugars found in the tested genotypes compared to glucose, sucrose, and fructose. FOS were inversely correlated with fructose, in particular under 60 % solar radiation reduction, which was partially evident with a 40 % reduction and absent under normal solar radiation conditions.

The distribution of the studied accessions along the PC1 and PC2 axes shows the first axis absorbs 27.80 % of the total variation while the second axis absorbs 26.82 % of the total variability (Fig. 10). PC1 emerges as a powerful discriminator, positively associated with antioxidant parameters such as TPC, DPPH, FRAP, and ORAC, while negatively correlated with TFC, FOS, and total sugars. Conversely, PC2 introduces a different perspective, emphasizing carbohydrate-related traits like TFC, sucrose, glucose, and fructose.

Group 1 (Green Circle - Fig. 10): Comprising BN at various growth stages and under different solar radiation (BN-SP-SR100, BN-MG-SR100, BN-BL- SR100, BN-SP- SR60, BN-MG- SR60, BN-BL- SR60, BN-SP- SR40, BN-MG-SR40, BN-BL-SR40). BN seems to lean towards traits associated with antioxidants, as evidenced by their significant positive correlation with PC1. This suggests a preference for characteristics linked to antioxidant activity over carbohydrates. Furthermore, within this group, distinctions emerge based on the stage of growth.

RU1-BL (Blue Circle): These genotypes present a more mixed profile, demonstrating correlations with PC2, particularly in carbohydrate-related traits. However, their preferences fluctuate across different growth stages. Notably, during the sprout stage, they display a consistent negative correlation with PC2, indicating a tendency to prioritize the accumulation of total sugars and FOS (Purple Circle in the graph). This intricate analysis of RU1-BL provides insights into how these specific genotypes within the RU1 respond to and prioritize different traits during various growth stages.

RU2 (Yellow Circle): RU2 appear to favour carbohydrate-related traits, as they exhibit a strong negative correlation with PC1. This suggests a penchant for characteristics linked to carbohydrates over antioxidant activities. Notably, during the baby-leaves stage in SR40, where the SR is at 40 % these genotypes show a high correlation with TFC, glucose, and sucrose, implying a tendency to prioritize the accumulation of these sugar types (Blue Circle in Fig. 10).

BN consistently leans towards antioxidants across solar radiation and different growth stages. The RU1, exhibits varying trait preferences. During the sprout stage, these genotypes tend to prioritize sugars and FOS. However, in the baby-leaves stage, their preference shifts towards fructose, glucose, and total flavonoid content (TFC). Notably, the growth stage significantly impacts the accumulation of these biochemical traits in the RU1. Conversely, RU2 consistently accumulates carbohydrates, especially during the baby-leaves stage, even when exposed to 40 % solar radiation. The data acquired showed different profiles among the studied genotypes in relation to solar radiation reduction. Specifically, RU2 sprouts and baby-leaves, exhibited significant variations in weight and dimensions of leaves under reduced solar radiation (SR40) compared to control conditions (SR100). For BN microgreens,SR40 showed the highest values of hypocotyl, cotyledon, and leaves dimensions. This insight could be valuable for enhancing plant productivity under specific conditions of limited solar radiation. For the biochemical profile, BN considering SR40, was positively correlated with antioxidant capacity, while RU1 and RU2 were linked to sugars, particularly glucose and sucrose. These findings could be useful for

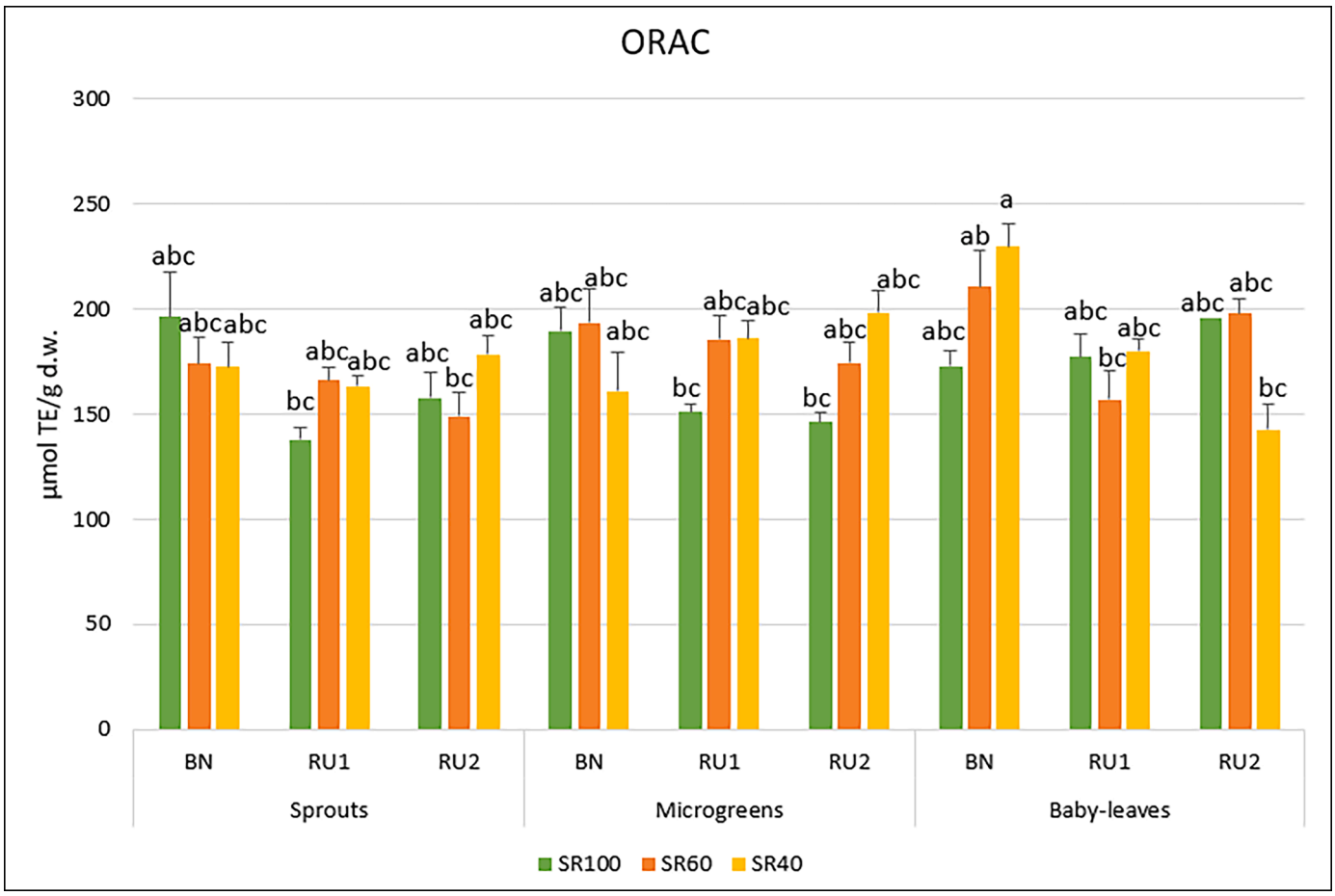

Fig. 2. Variation of ORAC (µmol TE/g d.w.) in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

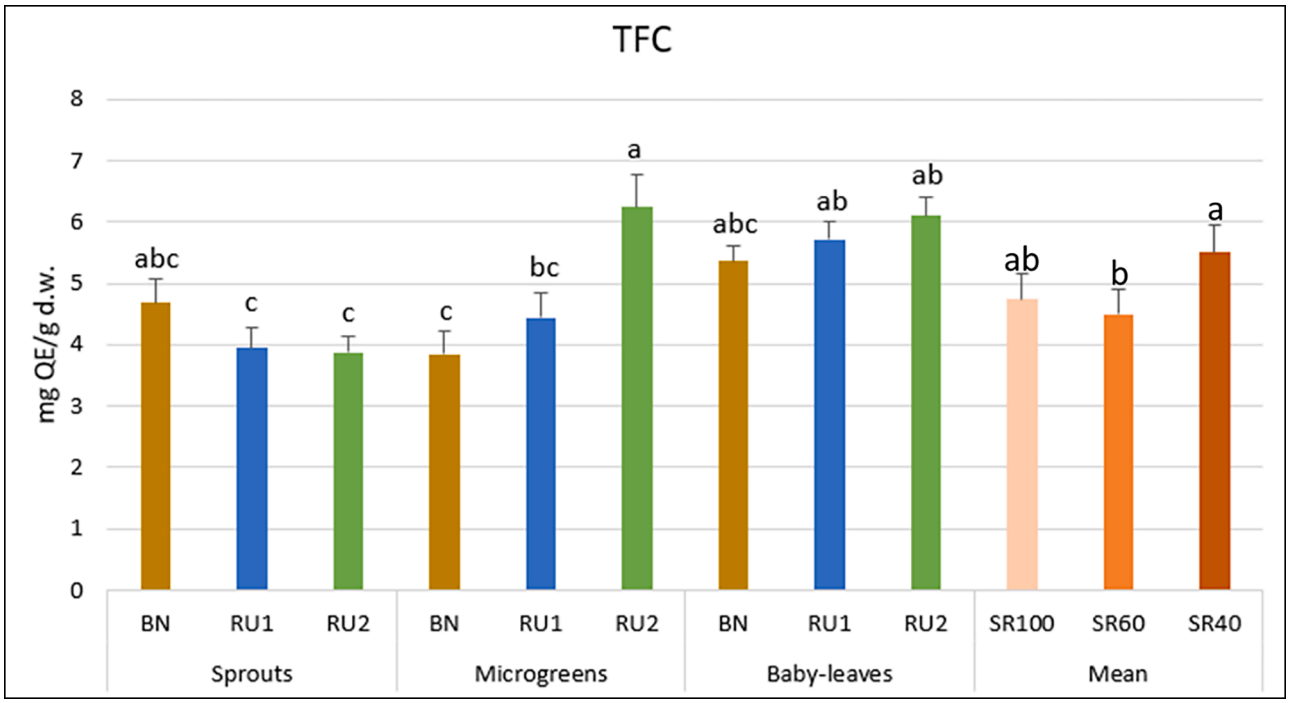

Fig. 3. Variation of TFC (mg Quercetin Equivalents/g d.w.) in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

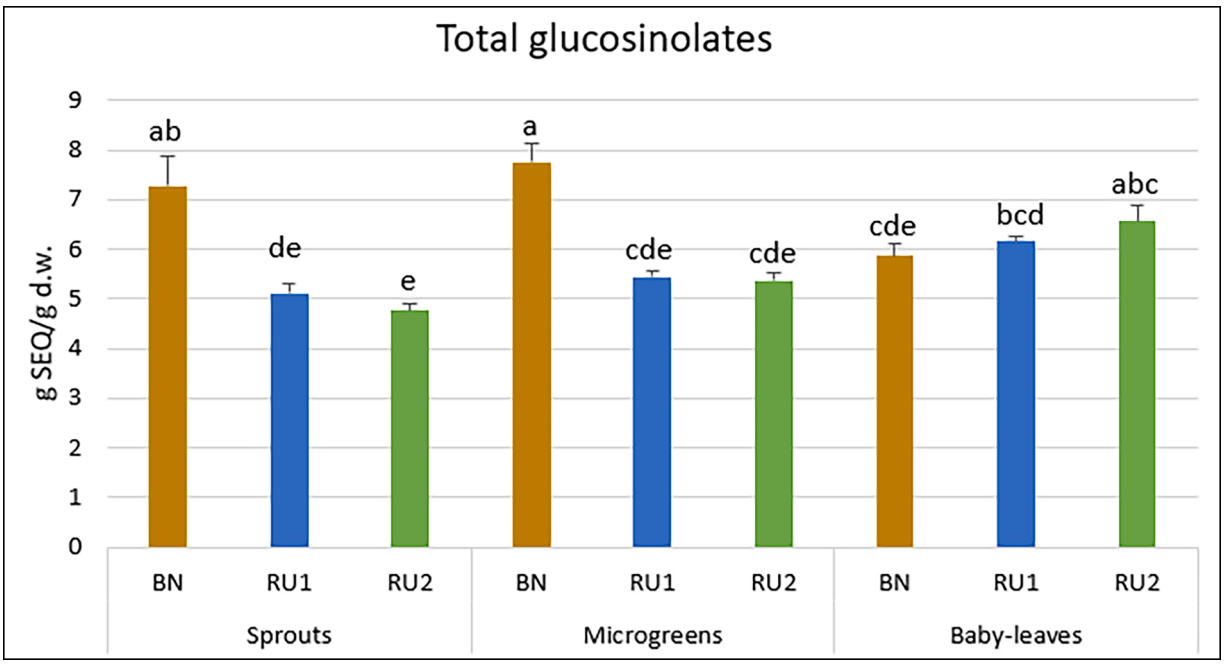

Fig. 4. Total Glucosinolates (g SEQ/g d.w.) variation in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

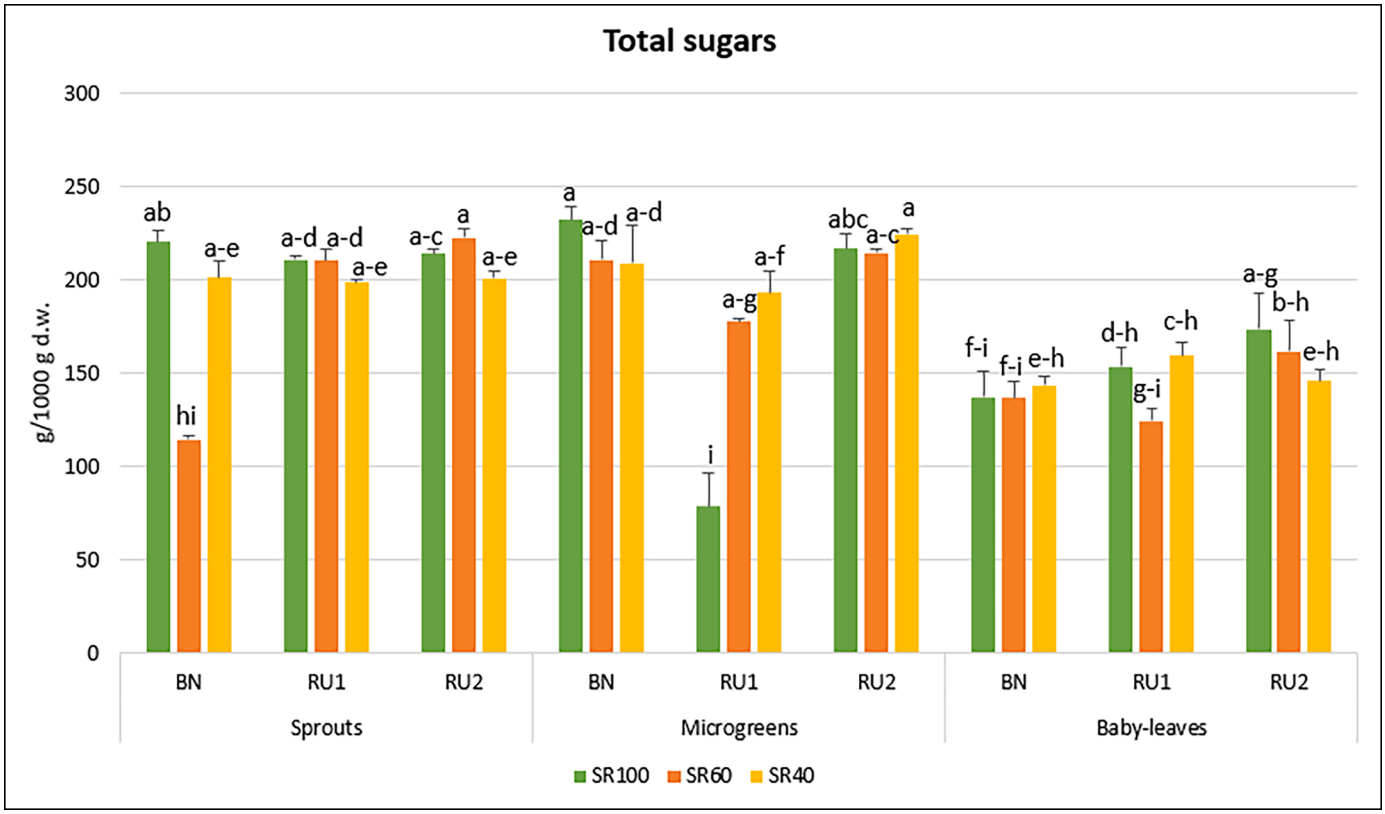

Fig. 5. Soluble Sugars (g/1000 g d.w.) in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

selecting the most efficient genotype, particularly in conditions of reduced solar radiation.

4. Discussion

The results acquired provide evidence of the effects of the variation of solar radiation on the morphometric and biochemical phenotyping of

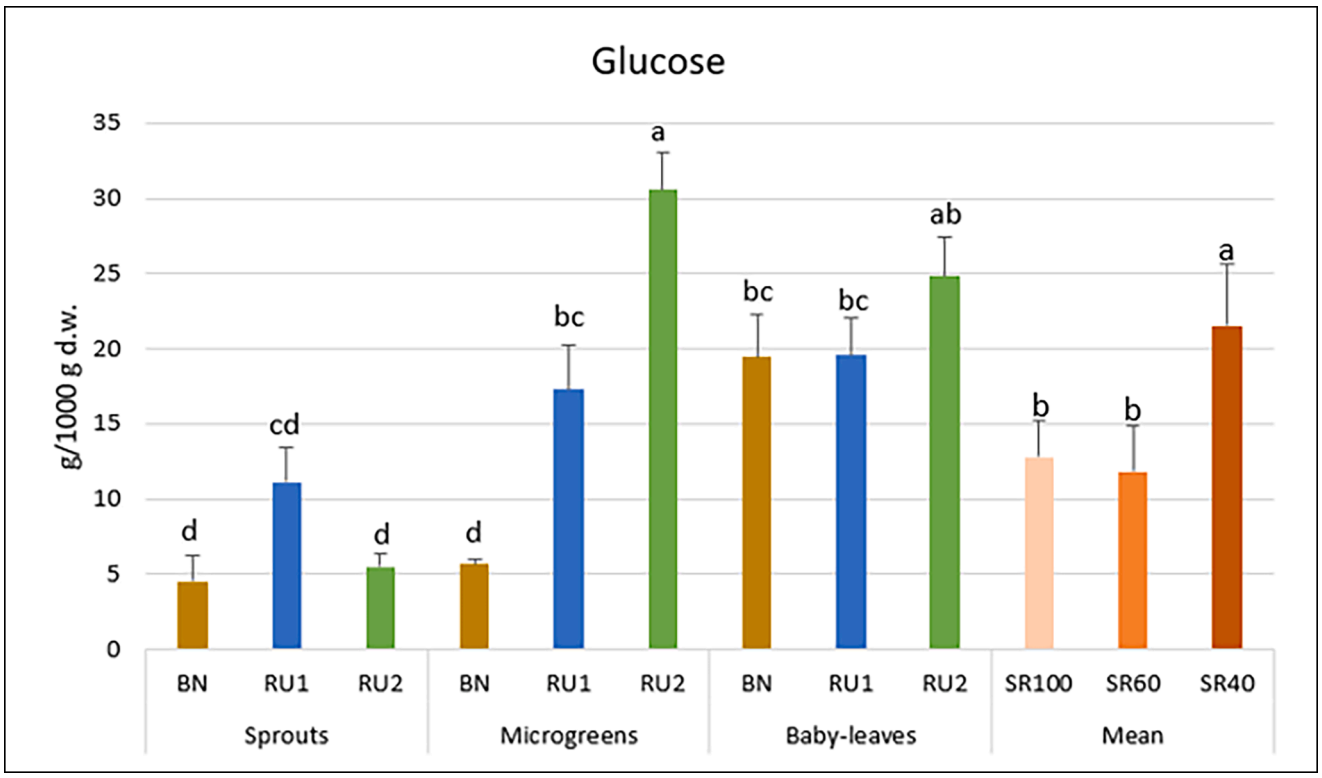

Fig. 6. The variation of glucose (g/1000 g d.w.) in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

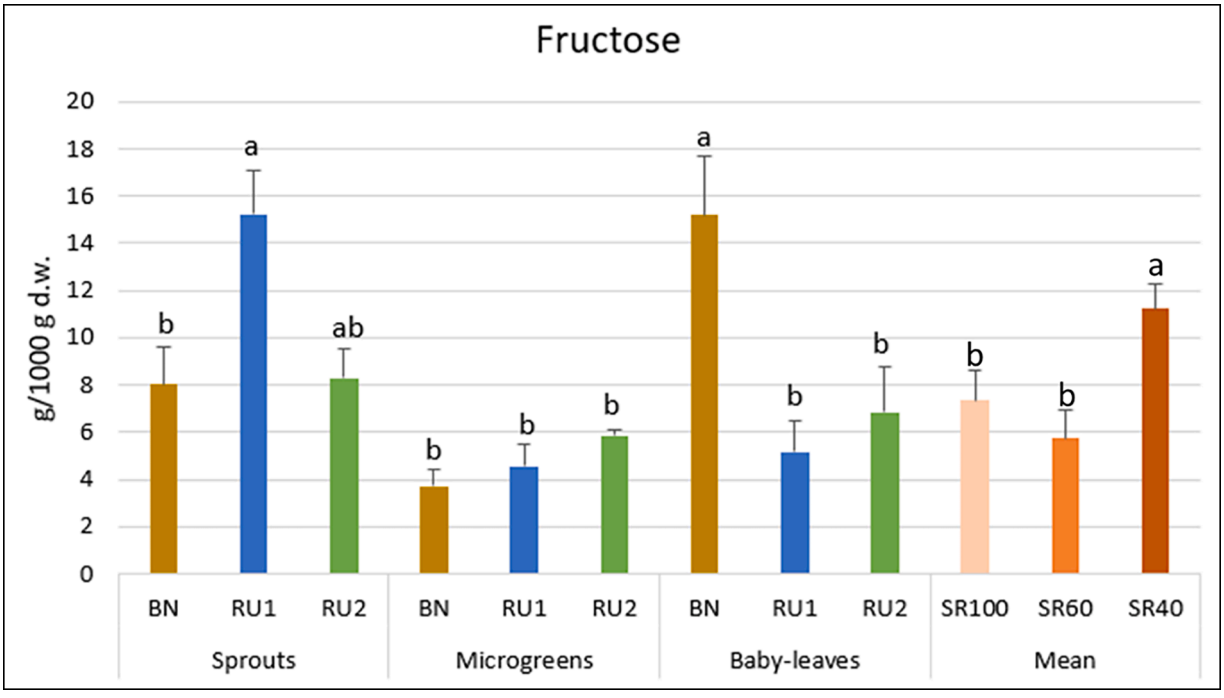

Fig. 7. Variation of the fructose (g/1000 g d.w.) in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

the plant. That will be useful for individuating genotypes of interest to grow in limited solar radiation conditions. Understanding how plants adapt to light reduction is pivotal for enhancing crop productivity and sustainability. The variations observed in growth characteristics among different genotypes and varying solar radiation conditions can be attributed to a combination of genetic factors and environmental influences. For sprouts, the weight of plants varied significantly with the reduction of solar radiation (SR40), with RU2 exhibiting the highest weight and BN displaying the lowest weight. For hypocotyl length, RU2 showed also the highest value, compared to the other genotypes.

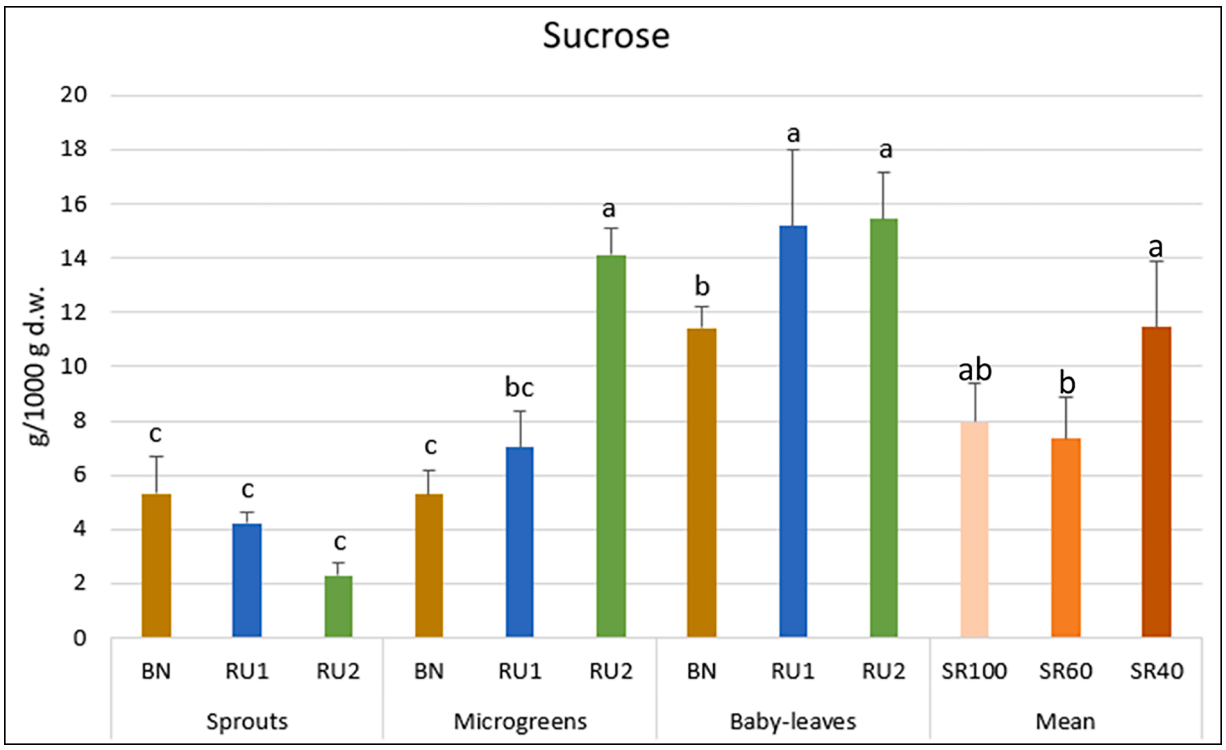

Fig. 8. Variation of the sucrose (g/1000 g d.w.) in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

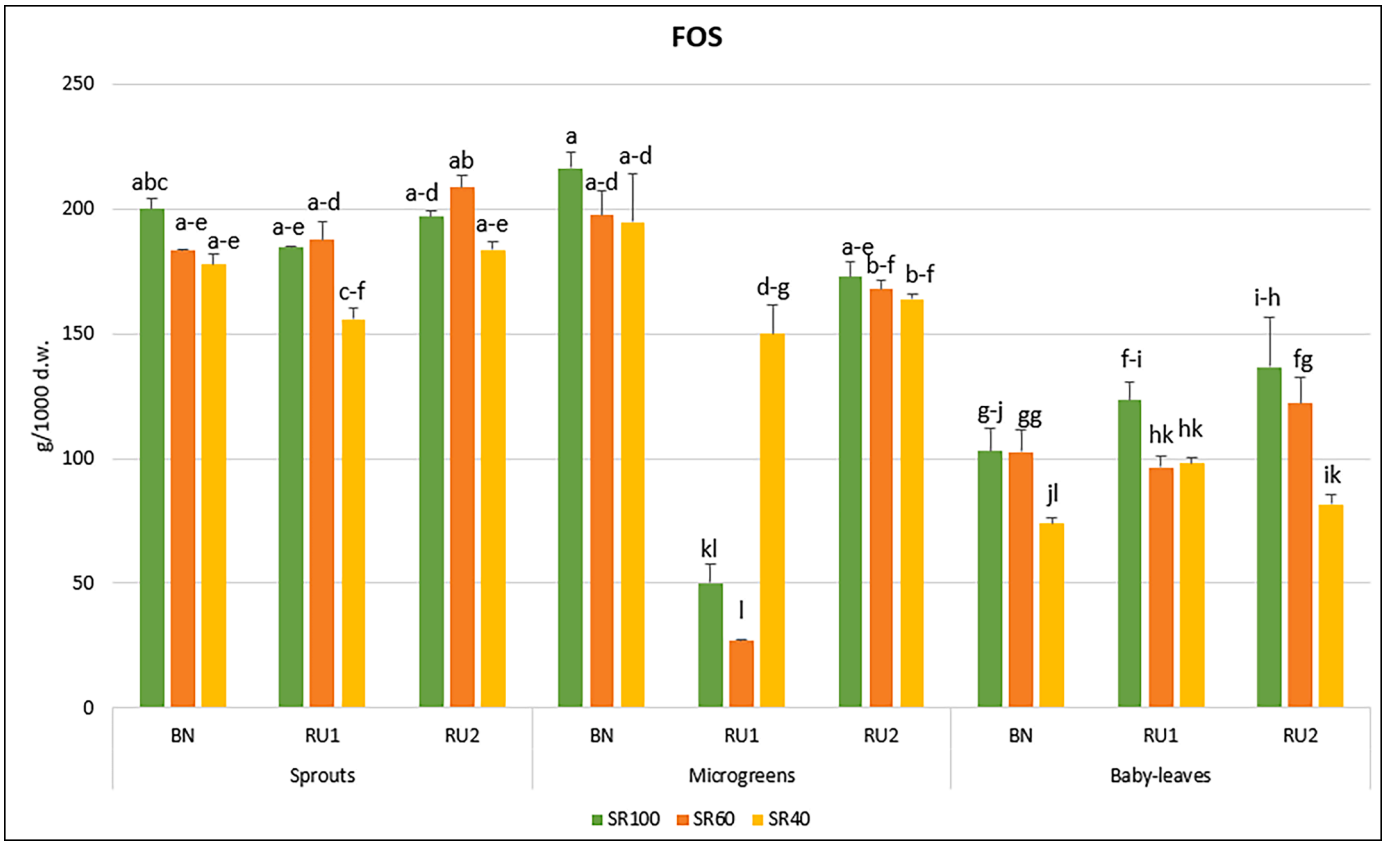

Fig. 9. Variation of the FOS (g/1000 g d.w.) in sprouts, microgreens, and baby-leaves of Broccolo nero (BN), *Eruca sativa* var. *darkita* (RU1) and *Eruca sativa* var. *lobata* (RU2). Each value represents the mean ± *S*.E. of three replicates. The letters above bars indicate significant differences according to Tukey's test ($p < 0.05$).

Table 5
Pearson's correlation coefficients among the different variables studied. TPC= Total Phenolic Content; DPPH= 1,1-diphenyl-2-picrylhydrazil scavenging; FRAP= Ferric Reducing Antioxidant Power; ORAC= Oxygen radical absorbance capacity; TFC= Total Flavonoid Content; TGLSs= Total glucosinolates content; FOS= fructooligosaccharides.

Correlation										
	TPC	DPPH	FRAP	ORAC	TFC	TGLSs	Total sugar	Glucose	Fructose	Sucrose
DPPH	**0.283***									
FRAP	**0.314****	**0.921****								
ORAC	**0.336****	0.214	**0.377****							
TFC	0.106	−0.160	−0.032	**0.249***						
TGLSs	**0.443****	**0.534****	**0.574****	**0.349****	−0.099					
Total sugar	−0.031	−0.074	−0.072	−0.038	0.055	−0.153				
Glucose	0.051	**−0.322****	**−0.274***	0.114	**0.549****	**−0.221***	0.072			
Fructose	0.092	0.146	0.107	0.018	−0.021	**−0.350****	0.175	**0.260***		
Sucrose	0.214	−0.138	−0.105	0.115	**0.628****	−0.017	0.036	**0.817****	0.095	
FOS	−0.070	−0.016	−0.022	−0.072	−0.105	−0.075	**0.953****	−0.216	0.017	**−0.220***

The interaction of the factors *, and ** indicate that the Tukey test is significant at $p < 0.05$ and $p < 0.01$ respectively.

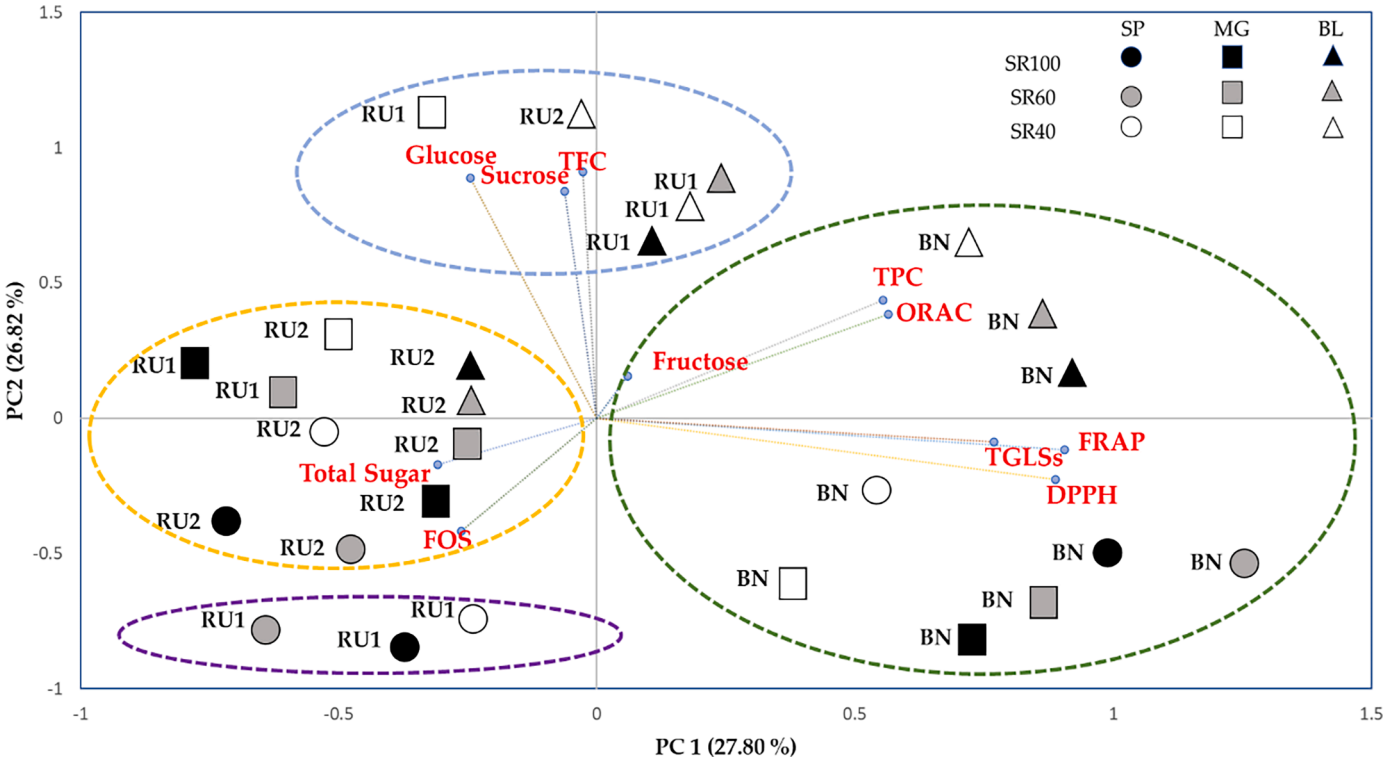

Fig. 10. Principal component analysis biplot with SP= sprouts, MG= microgreens, BL= baby-leaves, BN= Broccolo nero, RU1= *Eruca sativa* var. *darkita*, RU2= *Eruca sativa* var. *lobata*, TPC= Total Phenolic Content, DPPH= 1,1-diphenyl-2-picrylhydrazil scavenging, FRAP= Ferric Reducing Antioxidant Power, ORAC= Oxygen radical absorbance capacity, TFC= Total Flavonoid Content, TGLSs= Total glucosinolates content, FOS= fructooligosaccharides.

For microgreens, the weight (W) did not exhibit significant differences in relation to SR and GE. The reduction of solar radiation significantly affected the hypocotyl length, cotyledon length and width, and the dimensions of leaves. BN microgreens showed the highest values considering the reduction of 60 % of solar radiation (SR40). For baby-leaves, no significant variations were observed reducing the solar radiation. However, the stem length was significantly affected by the SR and GE. BN was the best performing genotype considering SR40. The number of leaves (N), varied significantly in relation to the genotype. A significant interaction was observed for leaf length (LL), with the highest values determined for RU2 SR40. Notably, the leaf width was also affected by the genotype, with BN displaying the best value. These findings highlight the complex relation between genetic factors and environmental conditions, particularly solar radiation, in shaping the growth and morphological characteristics of sprouts, microgreens, and baby-leaves. Such insights are critical for the development of strategies to optimize crop production under varying environmental conditions, ultimately contributing to enhanced agricultural sustainability and productivity.

The variations of solar radiation can determine a significant influence on plant growth and development through the selective activation of distinct photoreceptors (Folta and Carvalho, 2015). Furthermore, it should be noted that the effects of varying light quality differ among different plant species and varieties. In recent years, there has been a growing focus on the effect of light on plant research (Wang et al., 2023; Chiang et al., 2020). This shift in attention is driven by the recognition that green light can influence plant growth and the synthesis of internal substances through processes that involve cryptochrome-dependent and cryptochrome-independent pathways (Zhang and Folta, 2012). Changes in light availability can affect the rate of photosynthesis and the allocation of resources within the plant. This allocation determines the accumulation of biomass, including weight and stem length.

Light is a fundamental environmental factor affecting plant development. Solar radiation can modulate the expression of genes involved in photosynthesis, which, in turn, impacts plant growth and the production of secondary metabolites such as phenolic compounds and sugars (Pech et al., 2022). According to the study of Giorgetti et al. (2018), in the first 10 days of seedling growth, cotyledons consistently exhibited significantly higher Total Phenolic Content (TPC) compared to embryonic axes, with TPC peaking of 49.2 mg GAE/100 g d.w. at day 10. This content was approximately twice that of 4-day-old cotyledons, eight times higher than in 1–2-day-old tissues, and ten times more than in 40-day-old leaves. It is important to know that differences in phenolic contents among these studies and among different plant species can be attributed to a multitude of factors. These factors encompass variations in research methodologies, encompassing distinct approaches for extraction, chromatography, and quantification of phenolic compounds. Additionally, sample characteristics and conditions, such as growth and storage environments, have been recognized as influential variables in phenolic content variability (Bianchi et al.,2024). Our research aligns with this pattern of variability, highlighting differences between the genotypes BN, RU1, and RU2. Specifically, our findings showed that BN is the best-performing genotype for phenolic content, contributing to a deeper understanding of the phenolic variability within the studied cultivars. According to the literature data, the phenolic compounds (kaempferol, apigenin, ferulic acid), are one of the main bioactive compounds found in Brassica crops, and in particular during the sprouts stage (Velasco et al., 2011). Variations in gene expression, driven by genotype-specific regulatory elements, can lead to differences in biochemical pathways. Genes related to phenolic compound synthesis, sugar metabolism, and antioxidant enzymes may be particularly relevant in this context. During the first plant growth stage, sprouts undergo a rapid development of functional chloroplasts and start the process of photosynthesis.

The profile and quantity of glucosinolates tend to be higher during the initial plant growth stage, a trend consistent with findings from previous studies (Fahey et al., 1997; Farnham et al., 2005). It's worth noting that indolic glucosinolates can comprise over half of the total glucosinolate content in mature broccoli. As a result, young broccoli sprouts emerge as an optimal source for obtaining glucoraphanin (GRA). However, our findings diverge from previous research (Huseby et al., 2013; Perez-Balibrea et al., 2008), which reported a significant reduction in glucosinolates concentration in plants cultivated in darkness compared to those grown under light conditions. This underscores the impact of abiotic stress on glucosinolates content, as elucidated by the study conducted by Ben Ammar et al. (2023).

The results obtained highlight that the reduction of solar radiation significantly affected the total and the extractable sugar content, as evidenced by different values among different genotypes (BN, RU1, and RU2). The sugars content, generally, was higher with the 60 % of solar radiation reduction. The glucose, fructose, and sucrose content in general, increased from sprouts to baby leaves, while we observed a decrease in the FOS content from sprouts to baby leaves. This emphasizes the critical consideration of the growth stage when evaluating FOS content in plants, as it greatly impacts FOS measurements. The study also demonstrates the considerable impact of light stress, represented by solar radiation (SR), on FOS levels. As SR levels increase, substantial changes occur in FOS values among all genotypes and growth stages. This highlights the sensitivity of FOS regulation to light stress conditions, shedding light on the dynamic nature of FOS content in response to environmental factors. Additionally, it is noteworthy that genotype RU2 displays unique FOS dynamics distinct from both BN and RU1. This observation underscores the genotype-specific nature of FOS regulation, suggesting that different genotypes may have individual mechanisms for controlling FOS levels and for sugar metabolism and dynamic.

The Principal Component Analysis (PCA) reveals a notable discriminatory impact, highlighting the significant variations between biochemical traits and genotypes, elucidating their preference for either antioxidant-related traits or carbohydrate-related measures across various growth stages and solar radiation. Thus, environmental stressors, as reduction of solar radiation, can trigger stress responses in plants. In the SR100, TPC demonstrates moderate to strong positive correlations with various antioxidant capacity measurements such as DPPH, FRAP, and ORAC. These correlations indicate that as TPC levels increase, so does the antioxidant capacity of the samples. This underscores the importance of phenolic compounds in contributing to the antioxidant properties of the tested substances. In the SR60, TPC continues to show substantial positive correlations with key antioxidant capacity measurements, including DPPH, FRAP, and ORAC. This confirms the significance of phenolic compounds in maintaining antioxidant properties even under reduced light stress conditions. However, in the SR40 condition, TPC maintains strong positive associations, not with antioxidant capacity measurements, but with sugar components such as sucrose, glucose, fructose, and total sugars. These associations suggest a link between elevated TPC levels and increased sugar content under higher levels of light stress. It's important to mention that DPPH and FRAP consistently exhibit strong positive correlations across all levels of solar radiation. This suggests that these two assays provide largely redundant information regarding antioxidant capacity. Additionally, ORAC also displays strong positive associations with TPC in both SR100 and SR60 conditions, emphasizing its relevance to phenolic content. Furthermore, in all conditions, sucrose and glucose show strong positive correlations, highlighting their close association, while total sugars exhibit exceptionally strong positive correlations with Fructooligosaccharides (FOS) in both the SR60 and SR40 conditions, indicating a consistent relationship between these variables. Furthermore, the changes in correlations across the different growth stages can be attributed to the dynamic physiological processes and metabolic priorities of the plants as they progress from sprouts to baby-leaves and finally to microgreens. These findings provide valuable insights into the intricate biochemical mechanisms underlying the development of edible plants and offer a foundation for optimizing their nutritional quality. Additionally, several studies have shown that climate change could be affected by the solar radiation induced by CO_2 emissions (Ohunakin, et al., 2015: Pan et al., 2004). Climate events, such as precipitation, temperature, and solar radiation, may have a negative impact on plant yield, biomass, and development. Therefore, the current study could represent an interesting input to select and identify plant varieties resilient to different solar radiation conditions or with good adaptability in order to maintain or increase productivity, which is important to ensure food security.

In summary, in the present study, the evaluation was limited to three genotypes, which may represent a potential limitation of the experimental trial. Despite this, the findings are valuable for identifying and selecting the most effective genotypes for growth under conditions of reduced solar radiation. This knowledge is crucial for farmers, as it can guide the cultivation of crops in regions with varying solar radiation levels, ultimately enhancing agricultural productivity. Furthermore, these insights contribute to our understanding of genotype performance under abiotic stress, offering a foundation for future research and agricultural strategies tailored to specific environmental conditions.

5. Conclusion

In conclusion, the present study showed the effects of the variations of solar radiation reduction on the morphometric traits and biochemical profile of the initial plant growth stages, as sprouts, microgreens, and baby-leaves. The tested genotypes showed a valuable adaptability to low solar radiation (SR40), in particular RU2 during the sprouts and baby-leaves stages, and BN for microgreens. The variations observed in weight, hypocotyl length, cotyledon width, and dimensions of leaves underscore the significant impact of both genetics and solar radiation reduction on plant growth. The opposite changes in levels of FOS vs simple sugars resulted strongly affected by solar radiation. Moreover,

the phenolic profile, antioxidant capacities and sugars content highlight the genotype-specific regulation of these compounds, deepening our understanding of biochemical variability within cultivars. Additionally, the correlation between Total Phenolic Content (TPC) and antioxidant capacity underscores the role of phenolic compounds in plant defence mechanisms, with implications for plant resilience under different solar radiation reductions. These findings could be used to further identify and select other genotypes efficient under solar radiation reduction, analysing the mechanisms linked to genotype responses in relation to solar radiation. Ultimately, these results provide evidence for the development of strategies aimed at optimizing crop production in different environmental conditions. The climate change currently represents an important and significant scenario that could affect the environmental sustainability and the crop productivity. Identifying the genotypes resistant to grow with solar radiation reduction which exhibiting high morphometric parameter, as yield, productivity, antioxidant properties, and elucidating the mechanism for their resistance, could be useful to mitigate the impacts of climate change on agriculture and contribute to agricultural sustainability, paving the way for a more resilient and efficient agricultural future.

Supporting Information

The supporting information is composed by Figure S1 with the temperature registered during the growing cycle for SR100, SR60 and SR40; Pearson's correlation coefficients among the different variables studied for SR100 (Table S1, .doc file); Pearson's correlation coefficients among the different variables studied for SR60 (Table S2, .doc file); Pearson's correlation coefficients among the different variables studied for SR40 (Table S3, .doc file); Pearson's correlation coefficients among the different variables studied for sprouts stage (Table S4, .doc file); Pearson's correlation coefficients among the different variables studied for microgreens stage (Table S5, .doc file); Pearson's correlation coefficients among the different variables studied for baby-leaves stage (Table S6, .doc file).

CRediT authorship contribution statement

Donata Arena: Conceptualization, Software, Formal analysis, Investigation, Data curation, Writing – original draft. **Hajer Ben Ammar:** Software, Formal analysis, Data curation, Writing – original draft. **Nikola Major:** Methodology, Investigation. **Tvrtko Karlo Kovačević:** Formal analysis, Investigation. **Smiljana Goreta Ban:** Conceptualization, Methodology, Data curation, Validation, Writing – review & editing, Visualization, Supervision. **Simone Treccarichi:** Investigation. **Roberto Lo Scalzo:** Conceptualization, Methodology, Validation, Data curation, Writing – review & editing, Visualization. **Ferdinando Branca:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113408.

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