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Exploring strategies to growth wild turnip sprouts as healthy food

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Abstract

Background Brassicas (*Brassicaceae*) are recognized as excellent sources of nutrients and bioactive compounds. Among these, wild turnip (*Brassica rapa* L.), holds significant promising nutritional properties owed to its abundant glucosinolates and phenolic compounds. To enhance its potential values, the application of elicitors is crucial and good strategy prompting an enrichment in the concentration of phytochemicals, as well established in other relevant Brassicas, such as broccoli. While the responses triggered by certain elicitors such as salicylic acid, methyl jasmonate, or chitosan are widely documented, little is known about the impact of electrolyzed water, an economically viable elicitor. Through elicitation strategies, the aim of this work was to unravel insights into enhancing the phytochemical content of wild turnip sprouts for potential use as healthy food, comparing with well-studied broccoli as control of the experiments.

Results Our findings revealed that wild turnip exhibited a notable higher glucosinolate (GSL) contents (487–712 mg 100 g^{-1} D.W.), than in broccoli sprouts. Furthermore, the use of electrolyzed water (2 vol.) boosted the accumulation of glucosinolates with significant increase up to twofolds the content. Specifically, treatments with salicylic acid (250 µM) and electrolyzed water (2 vol.) favored the significant increase of mainly aliphatic GSL (progoitrin, PRO; gluconapin, GNA; glucobrassicin, GBN). On the other hand, natural antioxidants such as of the characteristic acylated cyanidins present in wild turnip sprouts were not affected by the elicitor treatments, indicative of higher tolerance to oxidative stress in wild turnip.

Conclusions These observations underlined the potential of using electrolyzed water in wild turnips as elicitor for GSL-enriched food ingredients. Further studies will be necessary to align with the broader goal of evaluating abiotic and biotic factors affecting the phytochemical composition in mature organs not only in germinating seeds and sprouts, for agricultural performance for quality and healthy foods purposes.

Keywords Edible wild plants, Brassicas, Glucosinolates, Anthocyanins, Healthy and sustainable food

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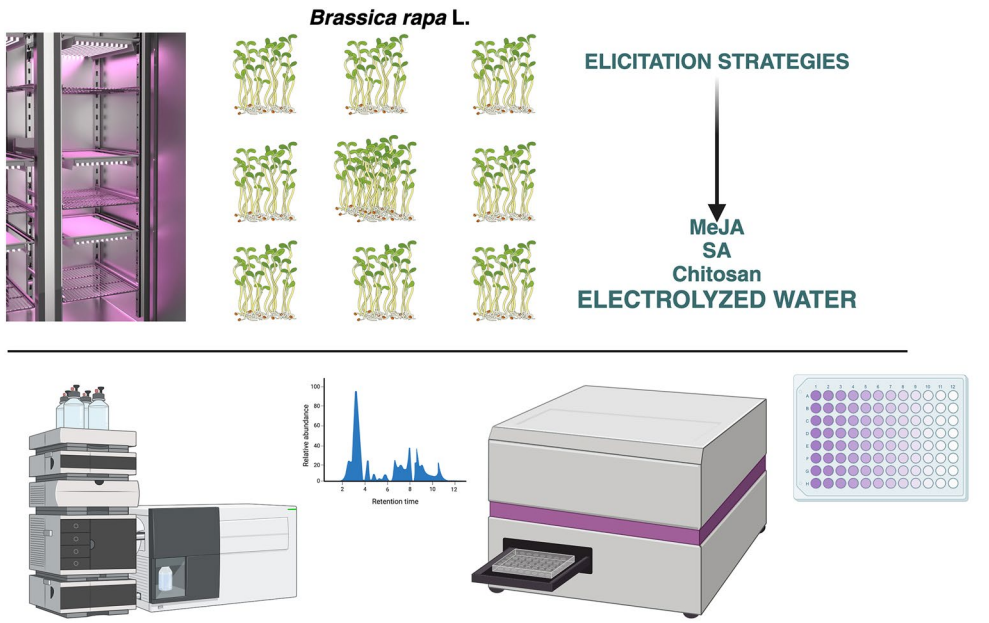
Graphical Abstract



Introduction

Brassicas are a group of plants belonging to the Brassicaceae family, which have gained significant attention due to their phytochemical composition and biological potential. These vegetables, including cabbages, broccoli, cauliflower, and mustards, are rich sources of bioactive compounds, such as glucosinolates, phenolic compounds, carotenoids, chlorophylls, and minerals [1]. The phytochemistry of Brassica species has been extensively studied, revealing the presence of various substances, including proteins, steroids, carbohydrates, phenols, terpenoids, flavonoids, and vitamins [2] and for their potential health benefits, particularly in the context of cancer prevention [3]. Some cruciferous vegetable, such as broccoli, contains high concentrations of health-promoting compounds, such as flavonoids, hydroxycinnamic acids, glucosinolates, and phenolic compounds [4–6]. These compounds have been associated with a wide range of biological activities, making Brassicas a subject of interest in various fields, including medicine, agriculture, and food science. Furthermore, the potential of Brassica species has been explored in improving metabolic health and mitigating obesity-related complications, highlighting their therapeutic applications in addressing metabolic disorders [7]. Nevertheless, the biological potential of the other Brassicaceae, such as wild turnip, is still unknown.

Wild turnip (*Brassica rapa* L.) is recognized for its opportunistic nature and as significant prospective for utilization as a food source due to its potential health benefits and nutritional value since it is rich in phenolic compounds, which are known for their potent antioxidative properties and potential protective effects against cancer and heart diseases [8]. In addition, wild turnip contains high amounts of glucosinolates, which have been associated with protection against pathogens, antimicrobial, and anticancer activities in humans [9]. Therefore, the recovery of wild turnip as a food source is interesting, making it a valuable addition to the diet. Hence, underutilized wild plants are highly relevant for food ingredients purposes. Their resilient nature can contribute to the establishment or valorization of these horticultural species for food security, promoting sustainable agriculture and exploring the nutritional and ecological potential of these often-overlooked plant species.

The elicitation in plants has acquired significant attention as a strategy to enhance the nutritional and functional properties of food products. Several elicitation strategies have been investigated for their potential to improve the bioactive compound content in various plant species. Methyl jasmonate, salicylic acid and chitosan

have demonstrated to be effective as biotic elicitors, to increase glucosinolate content in Brassicaceae sprouts, indicating the potential of elicitors in enhancing the nutritional quality of plants [10]. For instance, in some studies, it has been demonstrated that MeJA increased indole glucosinolates and their hydrolysis products while reducing goitrin in pak choi. In fact, the effect varied among cultivars, with changes also observed in primary metabolites, suggesting MeJA's potential for enhancing specific secondary metabolites [11]. The application of exogenous methyl jasmonate (MeJA) has been shown to increase the levels of certain glucosinolates (GSLs) in Brassica vegetables, such as neoglucobrassicin, glucobrassicin, and gluconasturtiin [12]. While MeJA treatment can enhance the nutritional quality of broccoli, it is important to remark that indole GSLs, particularly neoglucobrassicin, have been associated with negative health effects since it has been identified as highly mutagenic, and the consumption of indole-3-carbinol (I3C), a hydrolysis product of glucobrassicin, has been suggested to have dual properties where it may promote carcinogenesis under certain conditions [13, 14]. In addition, electrolyzed water has gained significant attention in the food industry due to its potential applications as a disinfectant [15]. Some authors emphasized the widespread use of electrolyzed water in the food industry for sterilization and safety, emphasizing its relevance as a disinfectant [16]. Electrolyzed water is considered as green technology which can be applied in seed decontamination, seed quality improvement and as an elicitor in plant systems, highlighting the use of $CaCl_2$–HCl electrolyzed water as a promoter of glucosinolates metabolism in broccoli sprouts through calcium signaling, indicating its potential role in eliciting beneficial plant responses [15]. As a result, electrolyzed water results to be an ecological and cost-effective alternative to other elicitors [16].

Therefore, the assessment of the phytochemical changes produced in wild turnip sprouts in response to elicitation treatments, emphasizing electrolyzed water, influencing the quality and composition of plant-derived foods, using broccoli sprouts as food model of brassica will contribute to sustainable food production and security.

Materials and methods

Reagents, chemicals, and standard solutions

Chitosan with 93% deacetylation and Mv = 140 000–220 000 g/mol, methyl jasmonate (MeJA) and salicylic acid (SA) were acquired from Sigma-Aldrich (Sigma–Aldrich, St. Louis, MO, USA). Electrolyzed water (Elect.W) was purchased from Hyposhield (Hyposhield, Puerto Montt, Chile), produced by electrolysis cell and based on hypochlorous acid (500 ppm), presenting a pH between 5.5 and 7.. Glucosinolates and anthocyanins standards were acquired from Sigma (St. Louis, MO, USA). Ultrapure water (18 MΩ cm) was produced using a Purist ultrapure water system from Rephile (Zhejiang, China). Acetonitrile and methanol, both HPLC grade, ethyl acetate, 2-butanone, and formic acid were obtained from Merck (Darmstadt, Germany). Trolox, fluorescein (free acid), and 2,2-azobis-(-2-methylpropionamidine) dihydrochloride (AAPH), 2,2.-diphenyl-1-picrylhydrazyl (DPPH) were acquired from Sigma Aldrich (St. Louis, MO, USA). Chromatography was performed on 20 cm × 10 cm HPTLC plates from Merck, coated with a 200 µm silica gel 60 F254. All the solvents used in the extractions were of analytical grade and obtained from Merck (Darmstadt, Germany).

Plant material and germination conditions

Wild turnip seeds ('Yuyo', *Brassica rapa* L.) were provided by Faculty of Agronomy (Chillán, Chile). On the other hand, commercial ready-for-sprouting seeds of broccoli (*Brassica oleraceae* L.) were provided by Semillería San Alfonso SL (Santiago, Chile), and were used as brassica control. The seeds underwent a rinse with distilled water and were then immersed in a 5 $g{\cdot}L^{-1}$ sodium hypochlorite solution under aeration for 24 h. Subsequently, after draining the soaking water, the seeds were weighed on day 0 and evenly distributed on trays (13.5 × 10.5 cm) with 5 g per tray, placed on coconut fiber as a substrate. Germination took place over 3 days in a controlled dark chamber at 25 °C to promote stem elongation of the sprouts. Following this initial phase, the trays were transferred to a controlled environment chamber with a 16-h light/8-h dark cycle, maintaining air temperatures of 25 °C during the day and 20 °C at night. The relative humidity (RH) was approximately 60% during the day and 80% at night. Photosynthetically active radiation (PAR) was set at 350 µmol m^{-2} s^{-1}. On day 10 after germination, specifically in the middle of the light period, six replicates per treatment of Brassicaceae sprout samples were swiftly and gently collected for analysis. All samples were weighed for fresh mass, collected separately, promptly frozen in liquid nitrogen, and stored at − 80 °C before analysis. Sprout length, fresh weight, and dry weight were determined for each treatment by measuring the distance between the base of the hypocotyl and the uppermost part.

Treatment with elicitors

The study selected electrolyzed water (2 vol.) as novel abiotic elicitor, and three classically used biotic elicitors: methyl jasmonate (MeJA, 50 µM), salicylic acid (SA, 250 µM), and chitosan (0.1 g L^{-1}). The concentrations of the used elicitorsin each assay were selected according

to a literature review [17–19]. Elicitors were applied as exogenous spraying on the cotyledons (not as soaking or irrigation solution) at 10 mL per tray from day 3 to day 10 of sprouting using Milli-Q water as a control. Six replicates were used for each treatment and species.

Extraction of bioactive compounds

Phytochemicals, including glucosinolates and anthocyanins, were extracted using various methods. Glucosinolates were obtained from freeze-dried samples (50 mg) by employing 1 mL of 70% (v/v) boiling methanol. The mixture was then subjected to heating at 70 °C for 30 min in a water bath, with intermittent shaking every 5 min. The extraction process was halted by placing the reaction mixture in an ice water bath for 5 min. Subsequently, the extracts were to centrifugate (17,500 g for 5 min), and the resulting supernatants were collected and filtered through a 0.45 µm PVDF filter. All samples were stored at − 20 °C until analysis.

For anthocyanin extraction, each sample (0.5 g) was initially combined with 5 mL of a solution consisting of 25:24:1 (methanol: water: formic acid v/v/v). The mixture was stirred or vortexed for 5 min, subjected to ultrasonication for 1 h, and left to stand overnight at 4 °C. Subsequently, the samples were centrifugated at 10,000 g for 10 min and filtered through a 0.22 µm PVDF membrane (Millex V13, Millipore, Bedford, MA, USA) before being transferred to amber vials for chromatographic analysis. All solvents employed in the extraction process were of analytical grade and sourced from Merck (Darmstadt, Germany).

HPLC–DAD–ESI–MSn analyses of glucosinolates and anthocyanins

Glucosinolates were identified through the examination of their UV–Vis spectra, retention times, and fragmentation patterns (ESI-ive, M- and MSn) using HPLC–DAD–ESI–MSn, following the methodologies described by [10]. Chromatograms were recorded at 227 nm, and the quantification of intact glucosinolates was conducted with glucoerucin and glucobrassicin as external standards for aliphatic and indolic glucosinolates, respectively (Sigma-Aldrich, St. Louis, MO, USA). The analysis was carried out in triplicate, and the results were expressed as mg 100 g^{-1} (DW).

For the analysis of anthocyanins, peak identification was conducted using the HPLC–DAD–ESI–MSn system (ESI + ive, M + and MSn), and previously established conditions for anthocyanins in cruciferous sprouts [5]. The quantification of the extracted samples was performed using a Hitachi HPLC–DAD system (Hitachi technologies, MERCK, Darmstadt, Germany) under the same chromatographic conditions, with chromatograms recorded at 520 nm, and using Cyanidin 3-*O*-glucoside as external standard (Sigma-Aldrich, St. Louis, MO, USA). The analysis was carried out in triplicate, and the results expressed in mg 100 g^{-1} (D.W.).

Antioxidant capacity colorimetric tests

The oxygen radical absorbance capacity (ORAC) assay [20] and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Mena et al., 2011) were employed to assess free radical scavenging activity. In the ORAC assay, the antioxidant capacity was determined by measuring the change in fluorescence after 120 min of the reaction with the radical. For the DPPH method, the evaluation was based on measuring the change in absorbance at 515 nm after 30 min of reaction with the radical. Both assays were conducted using 96-well microplates in a Synergy H1 hybrid multi-mode microplate reader (Biotek, Winooski, VT, US). The results were quantified and expressed as µmol Trolox 100 g^{-1} (DW). Each experiment was replicated six times.

Antioxidant capacity 'in silica' test: high-performance thin-layer chromatography (HPTLC) bioassay and mass spectrometry

The extraction methodology was developed following the method proposed by [21] with slight modifications. Briefly, the wild turnip and broccoli samples were homogenized in an analytical mill (IKA T25, Staufen, Germany). Then, 100 mg of the powdered sample was accurately weighed and transferred to a 2 mL tube. Thereafter, 1.5 mL of extraction solvent composite by ethanol: water: formic acid (50:49:1 v/v/v ratio) was added and vortex-mixed for 1 min. Ultrasound-assisted extraction was performed for 10 min at 39 °C using a Getidy (Wuyi Tongqin, China) ultrasonic bath with a frequency of 40 kHz and a potency of 40 W L^{-1}. Then, the suspension was centrifuged for 10 min at 9050×*g*, and the supernatant was filtered through a 13-mm polyvinylidene fluoride (PVDF) syringe filter (0.22 µm).

Samples solutions were applied by means of CAMAG (Muttenz, Switzerland) Automatic TLC Sampler 4 (ATS4), using the following settings: band length 6 mm, track distance 10 mm, dosage speed 120 nL s^{-1} and first application *x*-axis and *y*-axis at 10 mm. Application volumes for samples were of 10 µL, respectively. Chromatography was performed in a 20×10 cm twin-trough chamber (CAMAG) up to a migration distance of 70 mm for wild turnip and broccoli extracts using a mobile phase composed of ethyl acetate: 2-butanone: water: formic acid (7:3:0.8:1.2 v/v/v/v). The extracts were applied in duplicate, dividing the HPTLC plate into two sections: the first section was used for bioassay, and the second section was used for MS analysis.

After that, radical scavenging molecules were detected following the protocol described by [22] with some modifications. Briefly, the plate was sprayed with DPPH (1 mg mL^{-1}) dissolved in methanol. Then, the plate was dried at room temperature in the dark until white bands were visualized on a purple background, indicating the presence of antioxidant compounds. Finally, photo documentation was performed with white light (reflectance mode).

Following the methods reported by [23–25] (Aranda et al.; Galarce-Bustos et al.; Galarce-Bustos et al.),all molecules/bands exhibiting biological activity were analyzed by mass spectrometry. Inhibitory bands were selected and marked with a soft pencil on the HPTLC plate, based on the *h*RF value visualized under CAMAG cabinet at 254 and 366 nm. All antioxidant molecules were tentatively identified applying the following method: selected bands were eluted from the plate to MS by means of CAMAG TLC–MS interface using a mixture of methanol and acetonitrile (1:1 v/v) at a flow rate of 0.2 mL min^{-1} for 60 s. MS analysis was performed in Shimadzu (Kyoto, Japan) LCMS 8,030 triple quadrupole mass spectrometer with electrospray ionization (ESI) source operated with the following conditions: ESI in negative mode, capillary voltage 3.0 kV, nebulizing gas (N_2) 3 L min^{-1}, drying gas (N_2) 15 L min^{-1}, desolvation line temperature 250 °C, and block temperature 400 °C. Mass spectra were acquired in full scan mode between 50 and 2000 m/*z* values. Plate background signals were subtracted for each analysis. MS/MS analysis used argon as collision gas and collision cell voltages from − 20 and − 40 V. Data were acquired and recorded by Shimadzu LabSolution software version 5.51.

Statistical analyses

Data were collected and analyzed individually for each species. Significant differences in the results were assessed using Analysis of Variance (ANOVA), followed by the Tukey post hoc test. Statistical significance was considered at $p < 0.05$, and the analysis was conducted using R software (v. 4.1.0). For hierarchical clustering, the Euclidean distance method was employed, and the analysis was executed using the Morpheus tool 755 (https://software.broadinstitute.org/morpheus/). Principal Component Analysis (PCA) was conducted by mean-centering the data using eigenvalues to explore the correlation between variables and the discrimination of glucosinolate and anthocyanin contents for each species (wild turnip and broccoli sprouts), along with other characteristics. This analysis was carried out using R software, with the FactoMineR and ggplot2 packages. Results of ANOVA were included in Additional file 2: Tables S1 to S10).

Results

Growth performance

The physiological responses of wild turnip sprouts to elicitor exposure were also studied (Fig. 1A, B and C) to provide a complementary approach to bioactive compound experiments and compared to broccoli sprouts (Fig. 2A, B and C) exhibiting different responses to elicitor treatment.

These two species showed differences in hypocotyl length with broccoli doubling the sprout length compared to wild turnip (averaging 4.06 cm for the broccoli and 1.99 cm for the wild turnip). Regarding hypocotyl length, the different treatments had no significant effect on wild turnip ($p > 0.05$), but did affect broccoli sprouts. Broccoli sprouts were significantly longer at a concentration of 1 mg L^{-1} of chitosan and salicylic acid at a concentration of 250 µM, showing a 26% increase compared with the untreated control. Nevertheless, wild turnip and broccoli sprouts exhibited similar behavior in terms of weight. Chitosan was the elicitor that improved the fresh weight of wild turnip, presenting significant differences compared to the control treatment ($p > 0.05$). For broccoli sprouts, no differences were observed compared to the control. This could be explained by several factors, including the number of days of plant growth (10 days) and/or the type of substrate used (coconut fiber). The use of elicitors in Brassica sprouts has shown significant effect on biomass. Elicitors, such as methyl jasmonate, salicylic acid, and oligochitosan, have induced the accumulation of bioactive compounds, including glucosinolates and phenolic compounds, in various Brassica species, thereby influencing their growth and biochemical constituents [26–28]. In addition, a combination of blue LEDs, NaCl, and methyl jasmonate has been reported to enhance glucosinolate levels in mustard sprouts, indicating the interactive effects of elicitors and environmental factors on phytochemical accumulation, which can ultimately affect biomass [29]. These findings highlight the complex relationship between the use of elicitors and biomass in Brassica sprouts, emphasizing the potential of elicitation as a tool to modulate the bioactive properties and growth performance. In the present study, the effect produced by elicitors was biochemical, mainly affecting secondary metabolites, since no differences in length or weight were observed.

Glucosinolates

Wild turnip showed different glucosinolate profile (Table 1) compared to broccoli sprouts. Glucosinolates ranged from 487 to 711 mg 100 g^{-1} DW, doubling content in broccoli sprouts (Table 2) from 168 to 388 mg 100 g^{-1} DW.

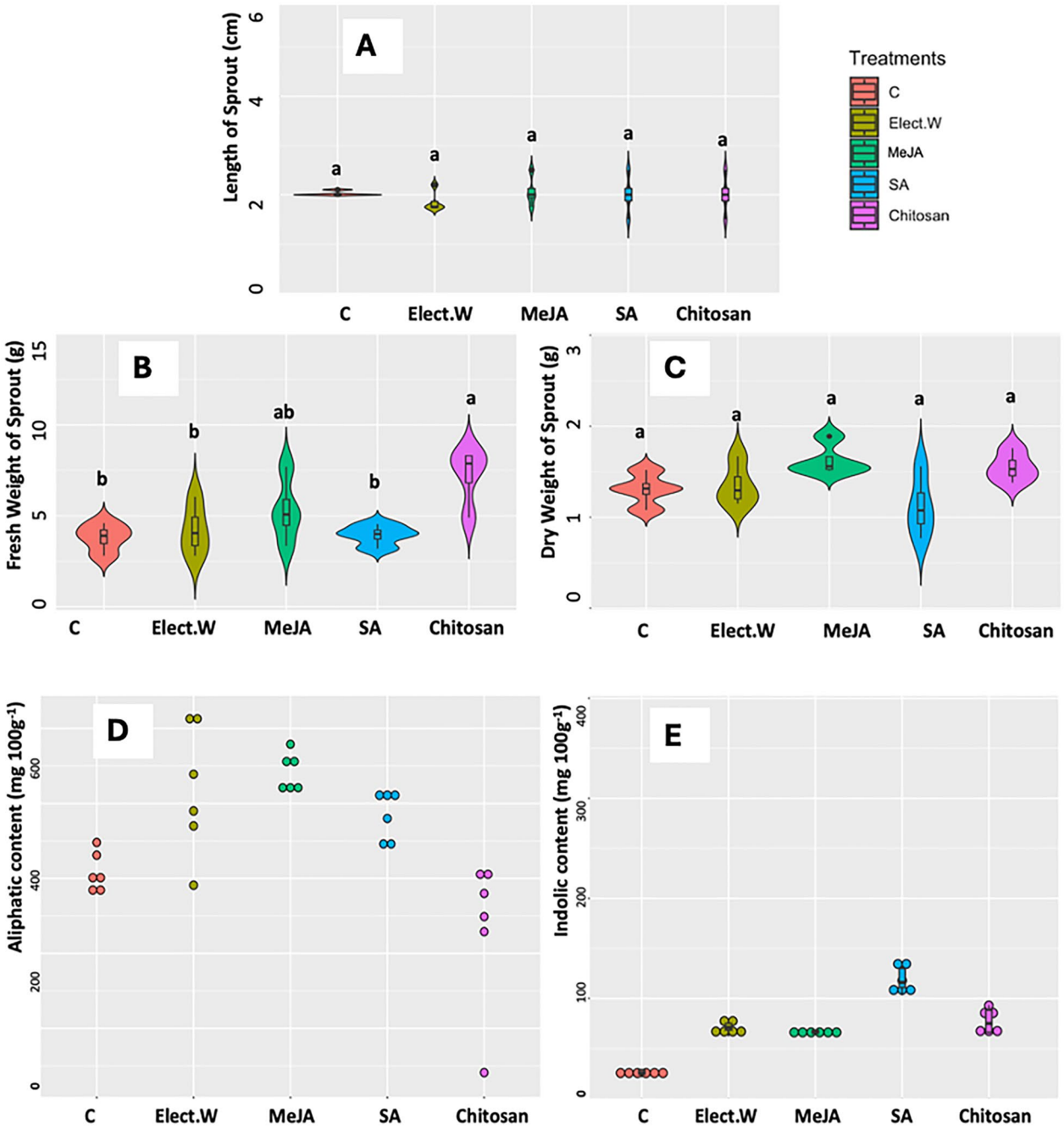


Fig. 1 Length (**A**), fresh weight (**B**), dry weight (**C**), aliphatic content (**D**) and indolic content (**E**) in wild turnip at different elicitor treatments C: control; Elect.W: electrolyzed water; MeJA: methyl jasmonate; SA: salicylic acid; Chitosan. Different letters mean significant differences at $p < 0.05$ in treatments for wild turnip sprouts analyzed according to Tukey test

In wild turnip, the total glucosinolate content significantly increased with the elicitor treatments compared to the control (28% superior), except for chitosan. The contents of individual glucosinolates (Table 1), progoitrin (PRO), gluconapin (GNA), hydroxyglucobrassicin (HGB), glucobrassicanapin (GBN), glucobrassicin (GBS), gluconasturtin (GNT) and neoglucobrassicin (NGB) were detected in wild turnips. Electrolyzed water showed a trend to increase the majority of the isolated GSL. In contrast, the exogenous application of chitosan decreased these values. The application of

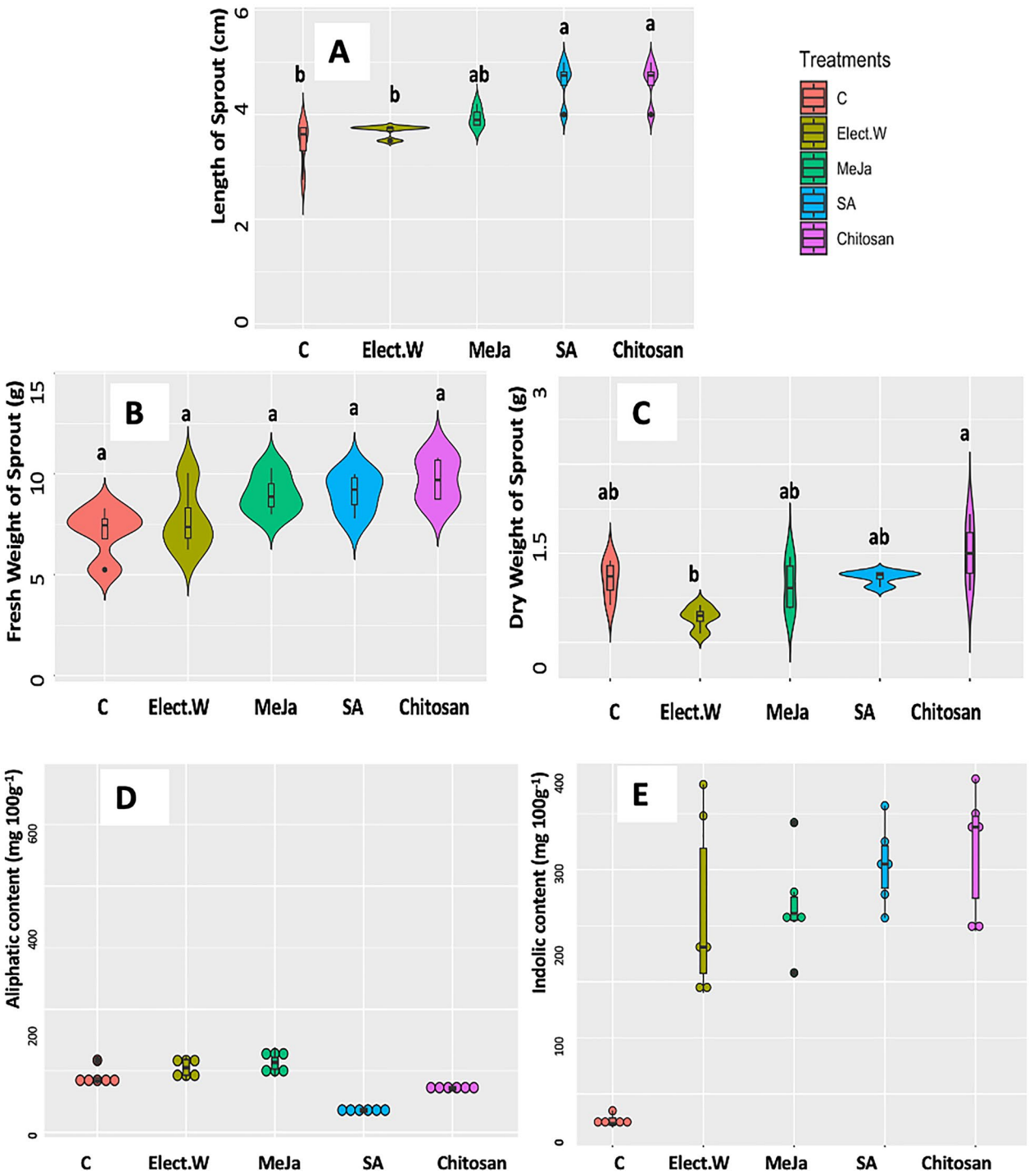


Fig. 2 Length (**A**), fresh weight (**B**), dry weight (**C**), aliphatic content (**D**) and indolic content (**E**) in broccoli at different elicitor treatments. C: control; Elect.W: electrolyzed water; MeJA: methyl jasmonate; SA: salicylic acid; Chitosan. Different letters mean significant differences at $p < 0.05$ in treatments for broccoli sprouts analyzed according to Tukey test

Table 1 Bioactive compound content (mg 100 g^{-1} DW) (± SD) in wild turnip sprouts after different elicitor treatments

	C	Elect.W	MeJA	SA	Chitosan
Glucosinolates					
PRO	9.02 ± 1.21 b	11.99 ± 3.01 ab	13.14 ± 3.35 ab	15.40 ± 4.18 a	9.30 ± 3.62 b
GNA	483.98 ± 29.17 a	586.84 ± 98.97 a	614.50 ± 31.94 a	532.85 ± 42.20 a	374.76 ± 94.18 b
HGB	0.09 ± 0.00 b	0.49 ± 0.06 a	0.13 ± 0.05 b	0.14 ± 0.06 b	0.13 ± 0.02 b
GBN	1.59 ± 0.21 a	1.54 ± 0.62 a	1.07 ± 0.44 a	1.38 ± 0.11 a	1.54 ± 0.42 a
GBS	15.42 ± 2.86 a	12.36 ± 3.90 a	14.93 ± 1.29 a	8.77 ± 1.65 b	9.57 ± 3.61 ab
GNT	20.63 ± 3.60 ab	39.02 ± 6.24 a	15.67 ± 1.23 b	30.68 ± 3.96 ab	22.59 ± 2.23 ab
NGB	11.01 ± 3.20 c	56.41 ± 3.52 b	51.84 ± 1.67 b	108.72 ± 12.34 a	69.00 ± 18.84 b
Total glucosinolates	541.75 ± 34.20 b	708.65 ± 89.61 a	711.27 ± 30.77 a	697.90 ± 43.11 a	486.89 ± 96.64 b
Anthocyanins					
5-Cy	0.20 ± 0.07 b	0.59 ± 0.26 a	0.28 ± 0.05 b	0.38 ± 0.08 a	0.47 ± 0.10 a
7-Cy	1.50 ± 0.54 a	2.14 ± 1.04 a	1.56 ± 0.49 a	2.03 ± 0.85 a	2.12 ± 0.89 a
8-Cy	0.20 ± 0.09 a	0.45 ± 0.16 a	0.20 ± 0.07 a	0.48 ± 0.18 a	0.33 ± 0.18 a
9-Cy	2.77 ± 0.72 a	3.10 ± 0.91 a	2.88 ± 1.25 a	3.17 ± 0.90 a	2.30 ± 0.20 b
Total anthocyanins	4.67 ± 0.67 b	6.28 ± 0.91 a	4.91 ± 0.31 b	6.06 ± 1.15 a	5.23 ± 0.40 b

C: control; Elect.W: Electrolyzed wáter; MeJA: Methyl Jasmonate; SA: salicylic acid; Chitosan; PRO is Progoitrin; GNA is Gluconapin; HGB is Hydroxyglucobrassicin; GBN is Glucobrassicanapin; GBS is Glucobrassicin; GNT is Gluconasturtin; NGB is Neoglucobrassicin; 5-Cy is cyanidin-3-O-(sinapoyl)(feruoyl)diglucoside-5-O-glucoside; 7-Cy is cyanidin-3-O(sinapoyl)(feruloyl)diglucoside-5-O-(malonylglucoside); 8-Cy is Cyanidin-3-O(sinapoyl)-diglucoside-5-O-(maloyl)glucoside; 9-Cy is Cyanidin-3-O(sinapoyl)-diglucoside-5-O-(maloyl)glucoside derivative. Different letters within the same row means significant differences at $p < 0.05$ in treatments for wild turnip sprouts according to Tukey test

Table 2 Bioactive compound content (mg 100 g^{-1} DW) (± SD) in Broccoli sprouts after different elicitor treatments

	C	Elect.W	MeJA	SA	Chitosan
Glucosinolates					
GRA	79.25 ± 16.75 ab	96.81 ± 19.03 a	99.32 ± 14.80 a	30.85 ± 3.65 c	61.44 ± 4.17 b
HGB	7.59 ± 2.66 b	17.27 ± 3.17 a	5.64 ± 0.44 b	2.07 ± 0.58 c	19.30 ± 6.81 a
GER	10.90 ± 3.00 b	7.95 ± 1.26 b	17.63 ± 5.55 a	3.82 ± 1.85 c	9.36 ± 2.07 b
GBS	24.48 ± 4.33 a	16.95 ± 2.78 b	26.44 ± 4.41 a	10.97 ± 1.90 c	13.66 ± 6.56 bc
MGB	34.60 ± 5.68 b	71.22 ± 13.93 a	20.59 ± 5.06 c	32.71 ± 10.15 bc	56.65 ± 5.93 ab
NGB	11.43 ± 3.40 b	181.10 ± 55.68 a	213.69 ± 55.27 a	258.94 ± 36.88 a	214.61 ± 61.66 a
Total glucosinolates	168.25 ± 16.21 b	387.81 ± 67.08 a	383.31 ± 56.82 a	338.11 ± 45.61 a	372.53 ± 59.88 a
Anthocyanins					
1-Cy	1.35 ± 0.64 ab	1.75 ± 0.46 a	1.54 ± 0.45 ab	1.05 ± 0.38 ab	0.49 ± 0.27 b
2-Cy	1.81 ± 0.45 ab	2.85 ± 0.79 a	1.79 ± 0.78 ab	2.15 ± 0.76 a	1.28 ± 0.71 b
3-Cy	0.37 ± 0.15 a	0.43 ± 0.13 a	0.37 ± 0.13 a	0.37 ± 0.03 a	0.24 ± 0.02 a
4-Cy	1.29 ± 0.37 a	1.09 ± 0.14 a	1.19 ± 0.67 a	1.26 ± 0.40 a	0.13 ± 0.03 b
5-Cy	3.56 ± 0.91 ab	3.67 ± 0.91 ab	4.79 ± 0.73 a	3.48 ± 0.93 ab	2.11 ± 0.91 b
6-Cy	0.70 ± 0.10 b	0.74 ± 0.12 b	3.06 ± 0.89 a	0.68 ± 0.21 b	0.55 ± 0.21 b
7-Cy	0.69 ± 0.20 c	3.42 ± 0.22 a	0.21 ± 0.03 c	2.76 ± 0.89 a	1.21 ± 0.38 b
8-Cy	1.42 ± 0.30 b	2.60 ± 0.78 a	1.45 ± 0.44 b	1.77 ± 0.21 ab	2.08 ± 0.37 a
Total anthocyanins	11.19 ± 3.52 ab	16.56 ± 2.33 a	14.40 ± 3.29 ab	13.51 ± 3.54 ab	8.08 ± 2.85 b

C: control; Elect.W: Electrolyzed wáter; MeJA: Methyl Jasmonate; SA: salicylic acid; Chitosan; GRA is Glucoraphanin; HGB is Hydroxyglucobrassicin; GER is Glucoeuricin; GBS is Glucobrassicin; MGB is Methoxyglucobrassicin; NGB is Neoglucobrassicin;1-Cy is Cyanidin-3-O-diglucosido-5-O-glucosido; 2-Cy is Cyanidin-3-O-(sinapoyl) diglucosido-5-O-glucsido;3-Cy is Cyanidin-3-O-(sinapoyl)diglucosido)-5-O-glucosido/cyanidin-3-O-(feruloyldiglucoside-5-O-glucoside (Coeluting); 4-Cy is Cyanidin-3-O(p-coumaroyl)(sinapoyl)diglucoside-5-O-glucoside; 5-Cy is cyanidin-3-O-(sinapoyl)(feruoyl)diglucoside-5-O-glucoside; 6-Cy is cyanidin-3-O(sinapoyl)(synapoyl) diglucoside)-5-O-glucoside; 7-Cy is cyanidin-3-O(sinapoyl)(feruloyl)diglucoside-5-O-(malonylglucoside); 8-Cy is Cyanidin-3-O(sinapoyl)-diglucoside-5-O-(maloyl) glucoside. Different letters within the same row means significant differences at $p < 0.05$ in treatments for broccoli sprouts according to Tukey test

salicylic acid increased the content of neoglucobrassicin (NGB) up to ten times higher than the control.

In broccoli, the total glucosinolate content significantly increased with elicitor treatment compared to the control (almost 50% superior). The individual glucosinolates contents, including glucoraphanin (GRA), hydroxyglucobrassicin (HGB), glucobrassicin (GBS), and methoxyglucobrassicin (MGB) were determined (Table 2). In this case, different responses were observed for each elicitor in isolation in relation to these various glucosinolates. Application of MeJA and electrolyzed water showed a trend to increase glucoraphanin content (GRA). Regardless, the results showed an increase in glucoraphanin content (GRA) after applying MeJA and electrolized water compared to the other treatments (SA and chitosan). Likewise, electrolyzed water increased the contents of HGB and MGB compared to control. The application of all elicitors increased neoglucobrassicin (NGB) content, reaching up to 20 times higher than the control. NGB is recognized as a stress indicator compound in broccoli because its content increases under abiotic stress condition [10]. GRA is the predominant glucosinolate in broccoli sprouts, representing almost 50% of the total glucosinolate content [10].

The Aliphatic glucosinolates content in wild turnip were higher that of indolic (Fig. 1D, E) and was also higher than the aliphatic glucosinolates found in broccoli (Fig. 2D). The aliphatic content ranged from 435 to 642 mg 100 g^{-1} and 35 to 113 mg 100 g^{-1} in wild turnip and broccoli. Whereas the indolic content found ranged from 25 to 119 mg 100 g^{-1} and 75 to 317 mg 100 g^{-1} in wild turnip and broccoli, respectively.

From these results, it is worth highlighting that the indolic content was always higher in the elicitation treatments than in the control for both the species. Interestingly, the indolic glucosinolate content was higher in broccoli than in the wild turnip. This type of compound tends to be synthesized to a greater extent when brassicas are stressed, so what was obtained here indicating that wild turnip is less stressed than domesticated broccoli.

Previous studies have shown that the application of sulfur as an elicitor in broccoli sprouts results in a dose-dependent increase in total glucosinolates, with higher dosages leading to greater enhancement [30]. In addition, phytohormones, such as methyl jasmonate, jasmonic acid, and salicylic acid, as well as oligosaccharides and amino acids, have been identified as effective elicitors for enriching sprouts with health-promoting glucosinolates [26]. Furthermore, elicitors have been found to improve the nutraceutical quality of broccoli sprouts, particularly in terms of the α-tocopherol and β-carotene levels [31]. Studies of MeJA treatment on *Brassica rapa* leaves led to metabolic changes, including reduced sugars and amino acids, increased hydroxycinnamates and glucosinolate, and prolonged accumulation of neoglucobrassicin and indole-3-acetic acid, suggesting a role in plant defense responses [32]Also, MeJA induced more and longer-lasting glucosinolate accumulation in Chinese cabbage compared to SA, with roots being more responsive, specifically for indole glucosinolates. On the other hand, neogluccobrassicin responded to MeJA in leaves and both SA and MeJA in roots, while 4-MGBC increased only in roots. Therefore, in this study, SA showed an antagonistic effect on MeJA-induced root glucosinolates [33].According to [34], the glucosinolate content in brassicas is affected by other environmental factors, such as temperature, radiation, and others. In that study, the researchers observed a decrease in the organosulfur bioactive content even before morphological and physical changes were observed in the plants and roots. The results obtained in this work indicate that the biochemical response in broccoli is faster than other mechanisms in plants, such as wild turnip.

Anthocyanins

Anthocyanins are crucial natural pigments for the organoleptic acceptance of foods, as well as interesting bioactive compounds in pharmacology and medicine. The levels of acylated anthocyanins in wild turnip sprouts were found to be lower than those broccoli sprouts after elicitation application, indicating a higher tolerance to oxidative stress in wild turnip (Tables 1 and 2). This observed phenomenon lies in the role of acylated anthocyanins and their relationship to oxidative stress response in plants, suggesting that wild turnip may have alternative or more efficient mechanisms for handling with oxidative stress, making it more resilient under such conditions.

Cyanidin 3-*O*-(sinapoyl)(feruloyl)diglucoside-5-*O*-(malonylglucoside) (from 1.50 to 2.14 mg 100 g^{-1}) and cyanidin 3-*O*-(sinapoyl)-diglucoside-5-*O*-(maloyl)glucoside derivative (from 2.30 to 3.17 mg 100 g^{-1}) were the most abundant anthocyanin in wild turnip whereas cyanidin 3-*O*-(sinapoyl)(feruloyl)diglucoside-5-*O*-glucoside was the most abundant cyanidin in broccoli, ranging from 2.11 to 4.79 mg 100 g^{-1}. The total anthocyanin content statistically increased after the application of electrolyzed water compared to control, for wild turnip, whereas, this application showed only a trend to increase for the broccoli.

The presence and abundance of highly acylated cyanidin derivatives in turnip was in agreement with previous reports in cruciferous sprouts [5]—including diglycosylated at C-3 and glycosylated at C-5 positions cyanidins, with of one or two cinnamoyl groups attached to the glycosylated fraction at position 3 (sinapoyl, feruloyl,

p-coumaroyl, and caffeoyl acylation) and malonyl at hexose in the position 5 (Tables 1 and 2). Total anthocyanins content ranged from 4.67 to 6.28 mg 100 g^{-1} and from 8.08 to 16.56 mg 100 g^{-1} in wild turnip and broccoli sprouts, respectively. Therefore, the anthocyanin content in broccoli sprouts was much higher than in the wild turnip sprouts. The effects of elicitors on sprouts may vary according to different situations as evidenced by the increase in total phenolic acids and antioxidant activity coupled with a decrease in total flavonoids in broccoli sprouts following the application of certain elicitors [28]. These findings highlight the complex interplay between elicitors, environmental factors, and phytochemical composition in sprouts.

Antioxidant capacity colorimetric tests

The anthocyanin content observed in both wild turnip and broccoli sprouts agreed with the antioxidant capacity assays, DPPH, and ORAC (Fig. 3).

The DPPH antioxidant capacity assay showed lower antioxidant capacity in wild turnip than broccoli (Fig. 3A, C). Wild turnip ranged from 874 to 1073 µmol Trolox 100 g^{-1} DW, whereas broccoli turnip ranged from 2485 to 4817 µmol Trolox 100 g^{-1} DW. In this assay, the activity observed in broccoli exceeded three times that of the wild turnip.

The values obtained in the ORAC assay showed similar behavior (Fig. 3B, D), ranging from 6388 µmol Trolox 100 g^{-1} DW to 7270 µmol Trolox 100 g^{-1} DW and from 15256 µmol Trolox 100 g^{-1} DW to 28128 µmol Trolox 100 g^{-1} DW in wild turnip and broccoli sprouts, respectively. Chitosan in broccoli showed the lowest antioxidant activity, which was consistent with the anthocyanin content. The antioxidant activity in broccoli exceeded three times that of wild turnip, indicating that wild plants were less stressed. Elicitors could trigger a stress response in broccoli and wild turnip plants by synthesizing more phenolic compounds (anthocyanins) with high antioxidant power. However, this was not observed in the ORAC assay.

The application of elicitors has been found to significantly affects the antioxidant activity of Brassica sprouts. Elicitors, such as methyl jasmonate and salicylic acid, have been shown to induce the accumulation of antioxidant compounds, including phenolic compounds, in various Brassica species, thereby influencing their antioxidant capacity [28, 35, 36]. Furthermore, the application of elicitors has been found to increase the total phenolic

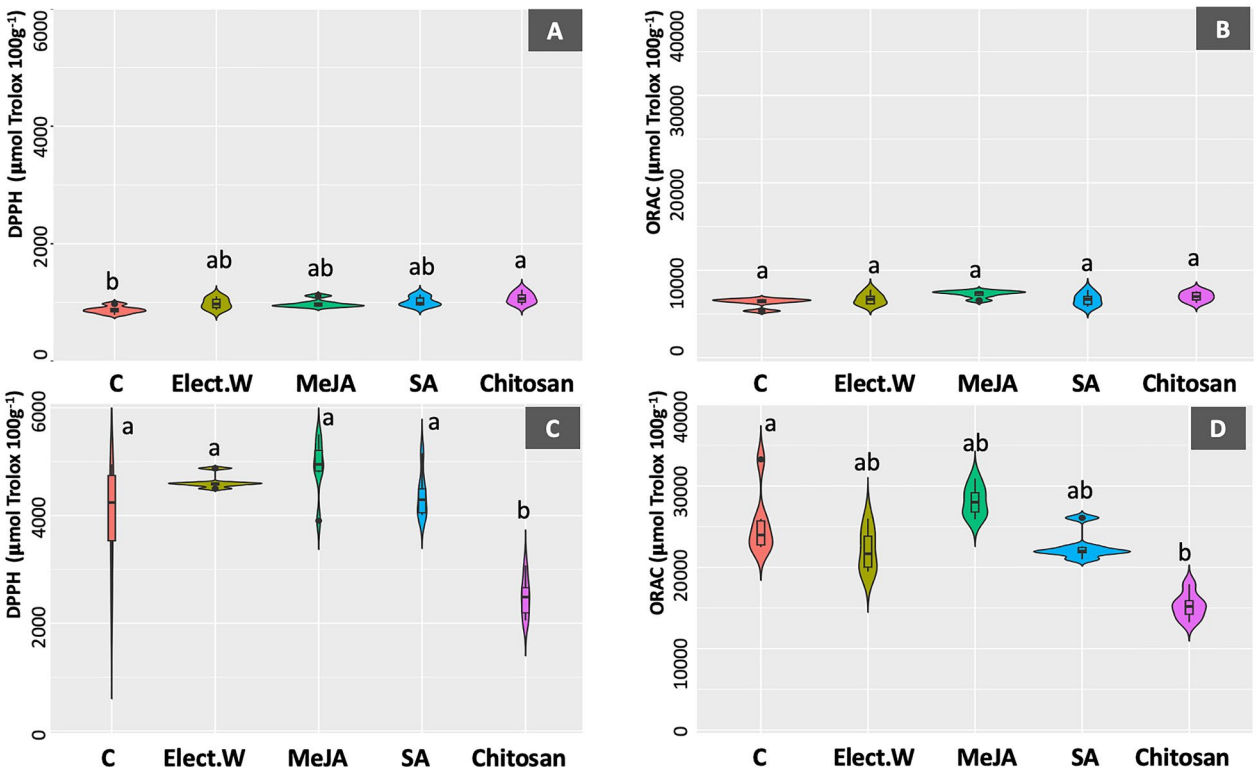


Fig. 3 Antioxidant capacity assays, DPPH in wild turnip (**A**) and broccoli (**C**) sprouts and ORAC in wild turnip (**B**) and broccoli (**E**) sprouts. *C* control; *Elect.W* electrolyzed water; *MeJA* methyl jasmonate; *SA* salicylic acid; Chitosan. Different letters mean significant differences at $p < 0.05$ in treatments for wild turnip and broccoli sprouts analyzed separately according to Tukey test

acid content and antioxidant activity of radish and broccoli sprouts, highlighting the potential of elicitation to modulate the antioxidant properties of these plants [28]. These findings underscore the correlation between antioxidant capacity and anthocyanin content in wild turnip.

The results showed the difference between domesticated and wild brassicas and their association with oxidative damage in plants. However, further studies are required to understand the specific mechanisms involved.

Antioxidant capacity 'in silica'

HPTLC chromatograms of anthocyanins from wild turnip and broccoli were conducted on silica gel F254 plates (Fig. 4). Detection of isolated antioxidant molecules was performed using HPTLC-bioassay and documented at white light.

As depicted in the figure, antioxidant broccoli compounds were observed as colorless bands on a purple background, with a higher intensity of colorlessness compared to wild turnip, indicating compounds with greater activity. These findings align with the results obtained from ORAC and DPPH tests conducted on wild turnip and broccoli extracts (Fig. 3) and the anthocyanin content obtained.

The bands showing the highest intensity were consistently present in all independent samples exposed to the respective treatment. Consequently, the samples used for the identification of compounds via mass spectrometry were those from the broccoli samples.

Mass spectrum of compounds detected can be found in Additional file 1: Figs. S1–S6). They allowed to characterize other compounds (different to anthocyanins) with high antioxidant activity, such as sinapoyl derivatives, flavonols and sinapic acid (*m/z* 753 [M—H]-; UV λmax = 269.6 nm), (*m/z* 959 [M—H]-; UV λmax = 282.9 and 300.6 nm), (*m/z* 899 [M—H]-; UV λmax = 278.5 nm), (*m/z* 591 [M—H]-; UV λmax = 282.9 and 300.6 nm), and (*m/z* 223 [M—H]-; UV λmax = 282.9 and 300.6 nm).

In this case, high-performance thin-layer chromatography (HPTLC) was followed by DPPH scavenging assay and mass spectrometry identification in brassica sprouts but other in vitro biological assays may be performance.

The application of HPTLC in Brassica sprouts has played a crucial role in both identifying and quantifying bioactive compounds, offering insights into their phytochemical composition [24, 25]. HPTLC has been utilized to assess the impact of elicitors on the bioactive composition of Brassica sprouts, specifically quantifying, phenolic compounds, and other phytochemicals [23]. For instance, studies have employed HPTLC to examine the influence of elicitors, such as methyl jasmonate and salicylic acid on the accumulation of glucosinolates in Brassicaceae sprouts, demonstrating the effectiveness of these elicitors in enhancing the bioactive profile of the sprouts.

Moreover, HPTLC has been applied to analyze the metabolic changes and elevated levels of bioactive

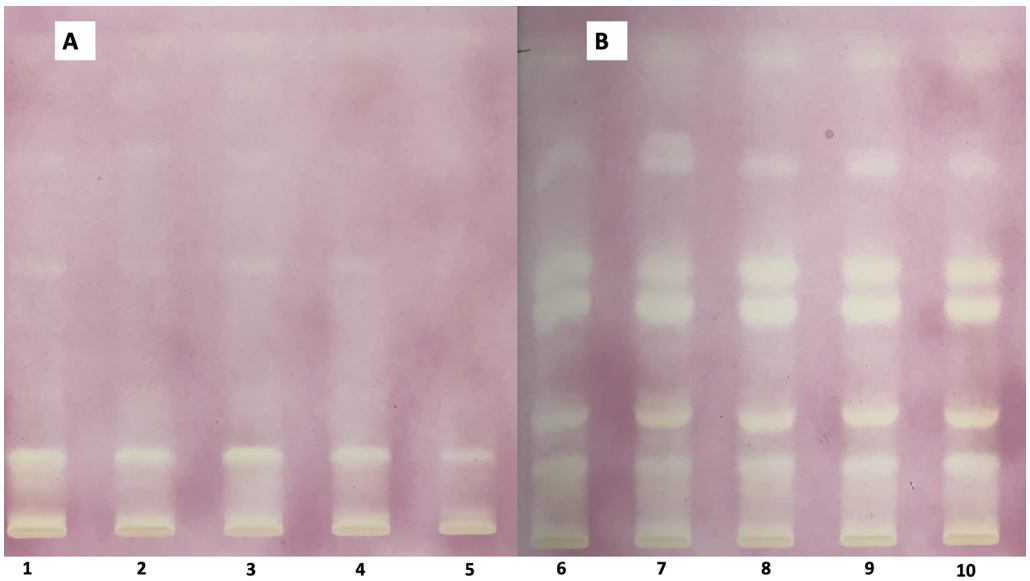


Fig. 4 HPTLC chromatograms of wild turnip (**A**) and broccoli (**B**) extracts on silica gel F_{254} plates. Detection of antioxidant molecules via HPTLC-bioassay photo-documented under 254 nm-UV, 366 nm-fluorescence, and white light. Selected bands (B1 to B6) for HPTLC–ESI–MS mass spectra analysis. GA control gallic acid. 1: control wild turnip; 2: MeJA wild turnip; 3: electrolyzed water wild turnip; 4: salicylic acid wild turnip; 5: chitosan wild turnip; 6: control broccoli;7: MeJA broccoli; 8: electrolyzed water broccoli; 9: salicylic acid broccoli; 10: chitosan broccoli

compounds in elicited white radish sprouts, providing valuable data on the phytochemical alterations induced by elicitation. In addition, HPTLC fingerprinting has proven to be a reliable tool for identifying and confirming the presence of various compounds in plant extracts [21, 22], highlighting its utility in characterizing the phytochemical profile of Brassica sprouts. These studies underscore the significance of HPTLC in elucidating the impact of elicitors on the phytochemical composition of Brassica sprouts, offering valuable insights into their bioactive properties.

Elicitation-mediated metabolic fingerprinting in wild turnip sprouts

Segregation based on the main glucosinolate and anthocyanin composition in wild turnip and broccoli was analyzed using a heat map (Fig. 5 and Additional file 1: 7S, respectively) and cluster analysis. The diverse patterns observed revealed distinct distributions among different elicitors and species.

Applications of electrolyzed water (Electro.W) to wild turnip exhibited a high content for all analyzed anthocyanins and glucosinolates. Interestingly, it was the only elicitor that demonstrated these results, followed by salicylic acid (SA). Glucosinolates GBS and GNA were associated with cyanidin 3-*O*(sinapoyl)(feruloyl)diglucoside-5-*O*-(malonylglucoside) (cysinferdiglumal) anthocyanins, while the remaining glucosinolates were grouped together. Only HGB and GNT glucosinolates, linked to stress in plants, were grouped with other anthocyanins. In contrast, broccoli displayed more variations among elicitors. Glucosinolates such as GRA, GER, or GBS were associated with Cyanidin 3-*O*-(sinapoyl)diglucosido-5-*O*-glucsido (Cysinsindiglu), while certain glucosinolates related to oxidative stress, such as HGB, MGB, and NGB, were grouped with other anthocyanins.

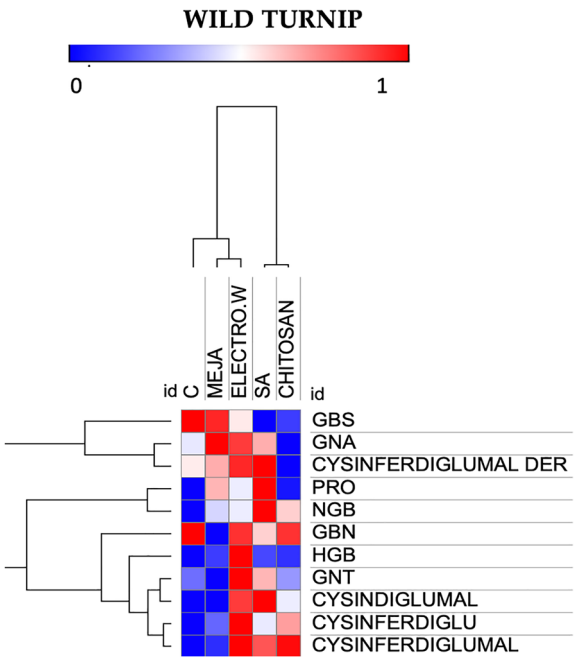


Fig. 5 Heat map and cluster analysis of main glucosinolates and anthocyanins in wild turnip at different elicitor treatments

Studies have employed cluster analysis and heat maps to assess metabolic changes and increased levels of bioactive compounds in elicited white radish sprouts, revealing clustering patterns of phenolic compounds and other bioactives induced by elicitation [27]. In addition, these techniques have been used to evaluate the impact of elicitation on the antioxidant and potential antihypertensive properties of lentil sprouts, providing a comprehensive visualization of the clustering of bioactive compounds and their correlation with bioactivity [37]. On the other hand, it is important to take into account the negative effect of these compounds and futher studies are necessary. In this sense, Haack et al.[38] suggested that neoglucobrassicin hydrolysis products inhibit GRA-induced NQO1 and GPx2 promoter activity via the AhR/XRE pathway, indicating a potential negative crosstalk between AhR/XRE and Nrf2/ARE pathways, limiting cancer chemoprevention by neoglucobrassicin-based glucosinolates.

Furthermore, the application of cluster analysis and heat maps has been pivotal in characterizing the effects of laser light on the accumulation of phytochemicals in Brassica sprouts, offering a comprehensive overview of the clustering patterns of primary and secondary metabolites induced by the treatment [39]. These studies underscore the significance of cluster analysis and heat maps in unraveling the complex interactions between elicitors and the phytochemical composition of Brassica sprouts, providing valuable insights into their bioactive properties.

Principal Component Analysis (PCA) was conducted for eight key traits in wild turnip and broccoli sprouts (Fig. 6 and Additional file 1: Fig. 8S, respectively). These traits included L (length of sprouts), DW (dry weight of sprouts), TGSL (Total glucosinolates), TA (Total anthocyanins), ALIPH (aliphatic glucosinolate content), INDOL (Indolic glucosinolate content), DPPH (DPPH assay), and ORAC (ORAC assay).

Examining the overall variability, PC1 retained 30.5% and 41.6% of the data variation for wild turnip and broccoli, respectively, while PC2 retained 27.3% and 26.9%, representing all traits as vectors in the biplot for both species. The length of vectors indicated how well the variables were represented in this plot. These results corroborate the findings of various assays.

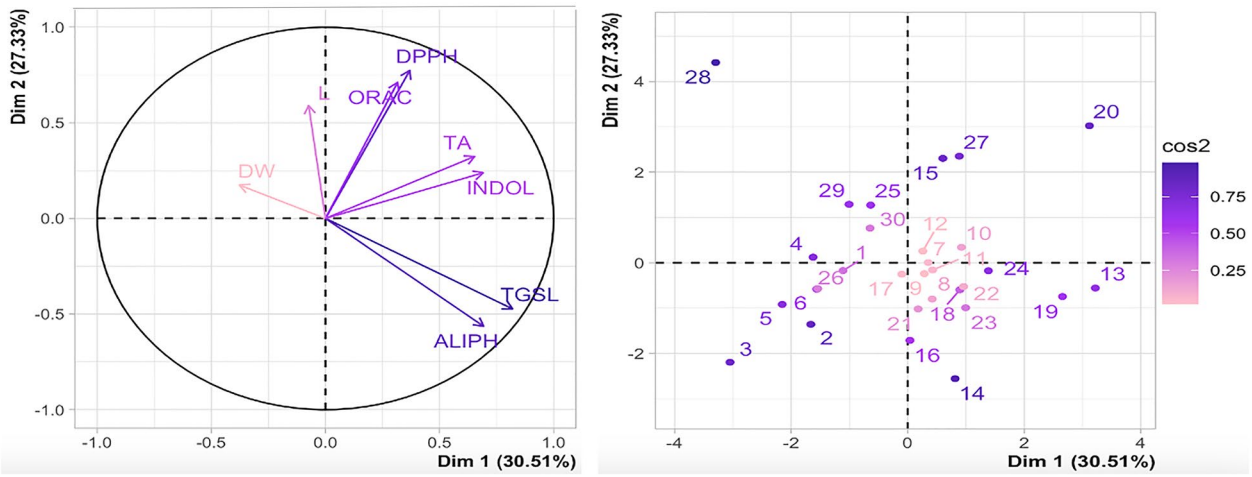


Fig. 6 Principal component analysis (PCA) of variables of wild turnip sprouts, *L* length of sprouts; *DW* dry weight of sprouts; *TGSL* Total glucosinolates; *TA* Total anthocyanins; *ALIPH* aliphatic glucosinolate; *INDOL* indolic glucosinolate; *DPPH* DPPH assay; *ORAC* ORAC assay

In the PCA of individuals, elicitor treatments for wild turnip and broccoli were represented by numbers 1 to 6 for the untreated control, 7 to 12 for MeJA treatment, 13 to 18 for Elect.W treatment, 19 to 24 for SA treatment, and 25 to 30 for chitosan treatment. Despite differences in elicitation strategies, the grouping indicated that samples responded similarly to variables based on the elicitation treatment, clustering close to the treatment that more intensively triggered the synthesis of bioactive compounds. MeJA, Elect.W, and SA induced greater synthesis of phytochemicals in wild turnip sprouts, while chitosan treatments induced higher biomass in sprouts. Similarly, in broccoli sprouts, these three elicitors induced a higher content of phytochemicals and antioxidant capacity, with chitosan resulting in greater length and dry matter. In both wild turnip and broccoli sprouts, untreated controls did not induce as intensive a response for these parameters.

Moreover, in broccoli, it was observed that TA, DPPH, and ORAC were highly induced by MeJA and Elect.W, whereas SA triggered higher total GSL and indolic compounds. This is attributed to abiotic stress promoting the production of anthocyanins, consequently increasing antioxidant capacity. The accumulation of anthocyanins is associated with antioxidant capacity and lipid peroxidation, all of which are involved in oxidative stress.

Untargeted metabolomic approaches, employing metabolic fingerprinting, have been utilized to evaluate the metabolic changes induced by elicitors in Brassica sprouts. This method provides valuable insights into the distinct impact of various elicitation treatments on the phytochemical profile of the sprouts [40]. These techniques play a crucial role in characterizing the clustering patterns of bioactive compounds and their relative abundance, illuminating the metabolic alterations induced by elicitors and their potential implications for the bioactive properties of Brassica sprouts.

Nevertheless, some experiments suggest that these elicitors effectively increased phytochemicals in wild turnip, further analysis is needed to optimize their application, as demonstrated in the case of MeJA in broccoli. In addition, the taste of the treated products may differ due to the elicitors, and adjustments in elicitor concentration levels should be based on sensory experiments, requiring further research. Previous studies using MeJA-treated broccoli showed a 50% increase in glucosinolates, improving nutritional quality, but it may also impact the raw taste due to neoglucobrassicin-derived compounds, while still maintaining taste in cooked or frozen forms [41].

Furthermore, the application of metabolic fingerprinting has proven instrumental in understanding the interplay between elicitation and the stimulation of specialized metabolic pathways, offering a comprehensive insight into the underlying mechanisms and their effects on gene regulation [42]. These studies highlight the significance of metabolic fingerprinting in unraveling the intricate interactions between elicitors and the phytochemical composition of Brassica sprouts, providing valuable insights into their bioactive properties and potential applications across various fields.

Conclusions

Synthesis of bioactive compounds in wild turnip sprouts were induced by the exogeneous application of elicitors. The content of glucosinolates in wild turnip was higher than in broccoli sprouts, possibly because wild turnip is less sensitive to changes although the

mechanism of tolerance under this situation needs additional work to evaluate. In fact, glucosinolate content in wild turnip was more than double than in broccoli, above all after application of elicitors as Elect.W.

Hence, the use of electrolyzed water, an economically feasible and easy-to-use elicitor treatment could be used in metabolite farming and biofactory strategies to obtain glucosinolate-enriched wild turnip sprouts for food and industry purposes. Exploring the mature organs of these wild and resilient crops merit further investigation.

With respect to the natural antioxidants in sprouts, the acylated anthocyanins in wild turnip were in lower contents than in the control broccoli sprouts after elicitation application, showing higher tolerance to oxidative stress by these plants.

The results open new to the use of elicitation as sustainable strategy in food crops, for resilient and healthy agricultural food production and human health.

Abbreviations

GSL	Glucosinolate
MeJA	Methyl jasmonate
SA	Salicylic acid
Elect.W	Electrolyzed water
AAPH	2,2-Azobis-(-2-methylpropionamidine) dihydrochloride
DPPH	2,2.-Diphenyl-1-picrylhydrazyl
ORAC	Oxygen radical absorbance capacity
HPTLC	High-performance thin-layer chromatography
RH	Relative humidity
PAR	Photosynthetically active radiation
PVDF	Polyvinylidene fluoride
HPLC–DAD-ESI-MSn	High performance liquid chromatography equipped with photodiode array detection-mass
ANOVA	Analysis of Variance
PCA	Principal Component Analysis
PRO	Progoitrin
GNA	Gluconapin
HGB	Hydroxyglucobrassicin
GBN	Glucobrassicanapin
GBS	Glucobrassicin
GNT	Gluconasturtin
NGB	Neoglucobrassicin
GRA	Glucoraphanin
GER	Glucoeuricin
MGB	Methoxyglucobrassicin
1-Cy	Cyanidin-3-*O*-diglucosido-5-*O*-glucosido
2-Cy	Cyanidin-3-*O*-(sinapoyl)diglucosido-5-*O*-glucsido
3-Cy	Cyanidin-3-*O*-(sinapoyl)diglucosido)-5-*O*-glucosido/ cyanidin-3-*O*-(feruloyldiglucoside-5-O-glucoside (Coeluting)
4-Cy	Cyanidin-3-*O*(p-coumaroyl)(sinapoyl) diglucoside-5-*O*-glucoside
5-Cy	Cyanidin-3-*O*-(sinapoyl)(feruoyl) diglucoside-5-*O*-glucoside
6-Cy	Cyanidin-3-*O*(sinapoyl)(synapoyl) diglucoside)-5-*O*-glucoside
7-Cy	Cyanidin-3-*O*(sinapoyl)(feruloyl) diglucoside-5-O-(malonylglucoside)
8-Cy	Cyanidin-3-*O*(sinapoyl)-diglucoside-5-*O*-(maloyl) glucoside
9-Cy	Cyanidin-3-*O*(sinapoyl)-diglucoside-5-*O*-(maloyl)glucoside derivative

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00576-y.

Additional file 1. Figure S1. Mass spectrum of compounds detected in broccoli samples (m/z 753 [M - H]-; UV λmax = 269.6 nm). **Figure S2.** Mass spectrum of compounds detected in broccoli samples (m/z 959 [M - H]-). **Figure S3.** Mass spectrum of compounds detected in broccoli samples (m/z 959 [M - H]-; UV λmax = 282.9 and 300.6 nm). **Figure S4.** Mass spectrum of compounds detected in broccoli samples (m/z 899 [M - H]-; UV λmax = 278.5 nm). **Figure S5.** Mass spectrum of compounds detected in broccoli samples (m/z 591 [M - H]-; UV λmax = 282.9 and 300.6 nm). **Figure S6.** Mass spectrum of compounds detected in broccoli samples (m/z 223 [M - H]-; UV λmax = 282.9 and 300.6 nm). **Figure S7.** Heat map and cluster analysis of main glucosinolates and anthocyanins in broccoli at different elicitor treatments. **Figure S8.** Principal component analysis (PCA) of variables of broccoli sprouts, *L*: length of sprouts; *DW*: dry weight of sprouts; *TGSL*: Total glucosinolates; *TA*: Total anthocyanins; *ALIPH*: aliphatic glucosinolate; *INDOL*: indolic glucosinolate; *DPPH*: DPPH assay; *ORAC*: ORAC assay.

Additional file 2: Table S1. ANOVA results of Length sprouts for wild turnip. **Table S2.** ANOVA results of Fresh Weight sprouts for wild turnip. **Table S3.** ANOVA results of Dry Weight sprouts for wild turnip. **Table S4.** ANOVA results of Total Glucosinolates for wild turnip. **Table S5.** ANOVA results of Total Anthocyanins for wild turnip. **Table S6.** ANOVA results of Length sprouts for broccoli. **Table S7.** ANOVA results of Fresh Weight sprouts for broccoli. **Table S8.** ANOVA results of Dry Weight sprouts for broccoli. **Table S9.** ANOVA results of Total Glucosinolates for broccoli. **Table S10.** ANOVA results of Total Anthocyanins for broccoli.

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Author contributions
MDLB: conceptualization, methodology, formal analysis, investigation, resources, visualization, writing. MTT: methodology, formal analysis, investigation. MI: methodology and formal analysis. KH, JFM: methodology, formal analysis, investigation. MS, NZ, SF, AP: conceptualization, methodology, investigation, resources, visualization, writing. CGV: conceptualization, writing review and editing. DAM: resources, conceptualization, methodology, writing review and editing.

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Availability of data and materials
The data set used and/or analyzed during the current study are available to readers as in the manuscript and from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not Applicable.

Competing interests
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.

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