Research Article

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Plant-derived biostimulant as priming agents enhanced antioxidant and nutritive properties in brassicaceous microgreens

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Abstract

BACKGROUND: Microgreens constitute dietary sources of bioactive compounds imparting numerous health benefits and enhancing sensory experience. They can be successfully cultivated in soilless systems where biostimulants can be easily integrated as seed-priming and post-germination agents improving the sustainability of a crop's final production. Compared to an untreated control, three priming agents (a commercial legume-derived protein hydrolysate (A250), a novel protein hydrolysate derived from peanut biomass (H250) and hydropriming (H_2O)) were applied to Komatsuna and Mibuna seeds grown as microgreens and compared for their effects on yield parameters, mineral composition, ABTS and FRAP antioxidant capacity, carotenoid concentration and phenolic compounds.

RESULTS: Significant effects of the main experimental factors and their interactions were identified on antioxidant capacity. Compared to the control and hydropriming, the highest ABTS and FRAP values were observed in Mibuna with the A250 and H250 treatments, respectively. Additionally, the H250 treatment increased the total concentrations of phenolic acid derivatives and flavonoid derivatives in Mibuna and Komatsuna, in tune with the levels of total flavonoids. Concerning mineral composition, the highest concentrations in both species were those of phosphorus and nitrate.

CONCLUSION: These results highlight the potential of select plant-based biostimulants as priming agents to enhance the antioxidant capacity, nutrient content and bioactive compound content, thus further increasing their functional and nutritive quality. In the light of this, the possibility of reducing the application of fertilizers by promoting a green transition for the intensive production of microgreens could subsequently be evaluated.

Supporting information may be found in the online version of this article.

Keywords: agricultural practices; bioactive value; Brassicaceae microgreens; carotenoids; polyphenols

INTRODUCTION

The World Health Organization considers healthy nutrition as one of the fundamental human rights. A healthy diet is a valuable tool to prevent many diseases and treat others. Specifically, a proper diet should be based on adequate macronutrient consumption to support metabolic energy demands, and reduced amounts of micronutrients (vitamins, minerals and bioactive compounds) essential for the proper functioning of physiological processes.[1] To achieve these goals, the consumption of saturated and trans fats, red meat, sodium-rich foods, refined carbohydrates and sugary beverages should be minimized or avoided altogether, choosing meals consisting of legumes, whole grains, fruits and vegetables.[2]

The drive toward a healthy lifestyle, which stems from a growing awareness of the link between nutrition and health, has increasingly sparked interest in emerging food products such as microgreens.[3,4] Recent estimates predict that the microgreens market will reach $17 billion by 2025, with a growth rate of approximately 8%.[5] Despite the lack of a clear legal definition for microgreens, they are currently defined as immature vegetables belonging to various botanical families (Brassicaceae, Lamiaceae, Apiaceae, Cucurbitaceae, Malvaceae, Asteraceae, Chenopodiaceae, Portulacaceae, Amaryllidaceae), harvested between the cotyledon stage and the appearance of the first true leaves (7–21 days after sowing).[6-8] Despite their small size (2–

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8 cm), microgreens are a powerhouse of nutrients (such as vitamins, minerals, fibers and phytochemicals), color and flavor,[4] giving them great potential in the food and beverage industry.[5] Their status as superfoods is attributed to their higher concentration of phytochemicals compared to the mature counterpart of a specific vegetable. To support this notion, studies by Hanlon and Barnes,[9] Huang *et al.*[10] and El-Nakhel *et al.*[11] have observed higher concentrations of phytochemicals and minerals in microgreens of radish (*Raphanus raphanistrum* subsp. *sativus*), red cabbage (*Brassica oleracea* L.) and lettuce (*Lactuca sativa* L.) compared to their mature counterparts, of which only edible parts are consumed. In contrast, microgreens are consumed in their entirety, without cooking processes that would inevitably lead to the loss of some of their nutraceutical properties.[4,12] To date, scientific research has focused on preharvest factors such as genetic material,[13,14] substrate selection,[15] nutrition management[16] and control of climatic parameters[17] to improve production and increase the concentration of beneficial phytochemical compounds. However, few studies have investigated the effects of biostimulant seed treatments on microgreens. Pannacci *et al.*,[18] Gaidau *et al.*[19] and Karbarz *et al.*[20] observed how the application of a plant-, animal- and microalga-based biostimulant, respectively, stimulated species-specific germination and hypocotyl elongation. Amirkhani *et al.*,[21] on the other hand, reported how coating seeds with a soybean-based biostimulant improved the growth of broccoli (*Brassica oleracea* L.) sprouts through an enhancement of nitrogen metabolism. Considering that microgreen production is moving toward a gradual reduction of chemical inputs to decrease residues in plant tissues,[22] the use of priming with biostimulant products seems to be the most suitable technique. Biostimulants have emerged as an innovation in recent decades, garnering much interest as they improve crop production and quality while significantly reducing the negative environmental impacts associated with indiscriminate agrochemical applications.[23,24] However, the effects of applying microbial and nonmicrobial biostimulant products on seeds are still underexplored, despite being an interesting topic of study due to their demonstrated positive effects on breaking seed dormancy, improving germination and enhancing seedling emergence.[25,26] On the contrary, the application of biostimulants to improve microgreen production and quality performance has rarely been explored for several reasons. First, the short crop cycle of microgreens may undermine the effects of biostimulant application, as multiple treatments are recommended at least on a weekly basis. Furthermore, foliar applications of biostimulants may cause mechanical damage to young seedlings and contaminate the final product. It was precisely for these reasons that our hypothesis was to use biostimulant products as priming agents, as the signal molecules present could trigger a positive action from the earliest stages (germination and cotyledonary leaf formation), thus overcoming the application problems described above. However, the application of biostimulants in a nutrient solution system without substrate in carrot and dill microgreens has shown very promising results.[7]

To further investigate the feasibility of integrating biostimulants into conventional microgreen cultivation, the experiment reported here utilized biostimulants as priming agents. This practice could represent an important crossroads for a more sustainable production model for microgreens that should include a reduction in chemical inputs in the future. From this perspective, the productive and qualitative responses of Mibuna (*Brassica rapa* var. japonica) and Komatsuna (*Brassica rapa* var. perviridis) microgreens to different priming treatments (two protein hydrolysates: a protein hydrolysate from peanut biomass and a commercial legume-derived protein hydrolysate (Trainer®)) were evaluated and compared to an untreated control and hydropriming.

MATERIALS AND METHODS

Growing conditions, plant material and harvesting

An experimental trial was conducted in the Department of Agriculture of Federico II University of Naples (Portici, Naples; latitude 40°49′11.6″ N, longitude 14°20′28.68″ E, with an elevation of 29 m above sea level) to evaluate the productive and qualitative response of two microgreen species to priming with biostimulant products. The trial was carried out in a controlled growth chamber equipped with an LED lighting system that provided a consistent intensity of 300 ± 10 μmol m^{-2} s^{-1} and a light spectrum ranging from 400 to 700 nm. The lighting parameters were adjusted using a portable spectroradiometer (MSC15, Gigahertz-Optik, Turkenfeld, Germany) at the canopy level. The photoperiod was set at 16 h of light and 8 h of darkness, while the air temperature and relative humidity were maintained at 26/18 °C (day/night) and 65–70/80%, respectively.

On 6 October 2021, Komatsuma (*Brassica rapa* var. perviridis) and Mibuna (*Brassica rapa* var. japonica) seeds (CN seeds, Pymoor, Ely, UK) were seeded in plastic trays (14 × 19 × 6 cm^3) filled with a peat substrate at a density of 5 seeds per square centimeter. The peat-based substrate (pH 5.48 and electrical conductivity (EC) of 282 μS cm^{-1}; Special Mixture, Floragard Vertriebs-GmbH, Oldenburg, Germany) had the following composition expressed in mg kg^{-1} dw: 11 nitrate, 140 phosphate, 796 potassium, 2402 calcium, 303 magnesium and 235 sulfate. Germination occurred in darkness at 24 °C and 100% relative humidity for all microgreen treatments, where only osmotic water was sprayed. Microgreens were placed in the growth chamber according to a randomized two-factor experimental design. The factors consisted of the two microgreen species (Komatsuma and Mibuna) and three seed priming treatments, namely hydropriming (H_2O), commercial legume-derived protein hydrolysate Trainer® (A250) and a new protein hydrolysate from peanut biomass (H250). Both protein hydrolysates were obtained through enzymatic hydrolysis as previously described by Colantoni *et al.*[27] An untreated control group (Control) was also included. Three days after sowing (DAS), the LED lighting system was activated, and the trays were rotated daily to ensure uniform light distribution. Throughout the growth cycle, a quarter-strength Hoagland nutrient solution with an EC of 0.3 dS m^{-1} and a pH of 6 was supplied to meet the nutritional needs of the microgreens, as previously described by El-Nakhel *et al.*[7] On a daily basis, fertigation was applied manually by means of a laboratory wash bottle instead of foliar spraying in order to avoid excessive humidity on microgreen stems and leaves after microgreen emergence (3 DAS). Irrigation volume ranged between 50 and 100 mL per tray. The exact volume depended on the growth stage, and the daily evapotranspiration of each tray was monitored in terms of the weight loss of each tray between irrigation cycles. Each experimental combination was replicated three times, resulting in a total of 24 experimental units ($n = 3$).

Upon the emergence of the first true leaf (14 DAS), the Komatsuma and Mibuna microgreens were harvested by cutting them at the substrate level. The harvested microgreens were then weighed to determine their fresh weight (fw) and the length of the hypocotyl was measured from the base of the seedling to the insertion point of the cotyledonary leaves. Furthermore, a portion of the harvested microgreens was immediately frozen at

−80 °C and subsequently lyophilized to analyze the concentration of minerals and bioactive compounds.

Priming treatments

Biostimulant products used for priming were two protein hydrolysates obtained by enzymatic hydrolysis of legumes[27]: the commercial product Trainer® (Hello Nature, Rivoli Veronese, Italy) and a new protein hydrolysate from peanut biomass. Both protein hydrolysates contained a similar amount of bioactive compounds such as peptides and amino acids (avg. 300 g kg^{-1}). The N and C content was also similar in both protein hydrolysates as follows: avg. 50 g N kg^{-1} and avg. 172 g C kg^{-1}. Because of the different sources of proteins, a different profile of bioactive compounds (peptides and amino acids) can be expected. Both biostimulants were used at a concentration of 250 mg L^{-1}. Furthermore, in the experiment, a control treatment with untreated seeds (Control) and a positive control treatment based on water priming (Hydropriming) were included. Before sowing, 10 g of seeds was weighed and immersed in 40 mL of the prepared biostimulant solutions (H250 and A250) and distilled water (H_2O). To avoid contamination, the vials were sealed with sealing film (Parafilm® M, Sigma-Aldrich, Milan, Italy) and placed in a rotary shaker in the dark at 20 °C for 24 h. After 24 h seeds were washed with distilled water and dried at their original seed weight in a desiccator (SICCO Mini 1, Bohlender, Grünsfeld, Germany) containing silica gel.

Determination of mineral concentrations

The method previously described by Formisano *et al.*[28] was followed to determine the anion (NO_3^-, P, S) and cation (K, Ca, Mg) concentrations. In brief, 0.125 g of finely ground lyophilized microgreen material was extracted in 25 mL of ultrapure water. Following the extraction steps (water bath at 80 °C with agitation for 10 min and centrifugation for an additional 10 min), the obtained supernatant was collected, filtered and processed using ion chromatography (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA) coupled with a conductivity detector. The integration and quantification of anions and cations were performed by comparing the peak areas of the samples with those of standards. Each treatment was analyzed in triplicate and the mineral concentrations were expressed in g kg^{-1} dw, except for the nitrates expressed in mg kg^{-1} fw.

Determination of antioxidant capacity

A ferric reducing antioxidant power (FRAP) assay was carried out following the protocol detailed by Rajurkar and Hande.[29] Briefly, 150 µL of each sample was mixed with 2850 µL of FRAP solution composed of 1.25 mL of 10 mmol L^{-1} TPTZ solution in 40 mmol L^{-1} HCl + 1.25 mL of 20 mmol L^{-1} $FeCl_3$ solution in water + 12.5 mL of 0.3 mol L^{-1} acetate buffer, pH 3.6, and then incubated for 4 min. After the incubation, the absorbance at 593 nm was measured. $ABTS^+$ antioxidant capacity was determined following the protocol described by Re *et al.*[30] After filtration and dilution (1:10) with 70% methanol, 0.1 mL of each sample was mixed with $ABTS^+$ solution. The resulting solution was stored for 2.5 min at room temperature, and then the absorbance at 734 nm was immediately recorded. Absorbance measurements for both assays (FRAP and ABTS) were performed using a UV–visible spectrophotometer (Shimadzu, Japan). All analyses were performed in triplicate and the results were expressed as mmol Trolox equivalents kg^{-1} dw.

Determination of carotenoid concentrations

The determination and quantification of carotenoids (*β*-carotene and lutein) were carried out by high-performance liquid chromatography with diode array detection (HPLC-DAD) in accordance with the method previously described by Salomon *et al.*[31] Specifically, 0.1 g of lyophilized plant tissue was extracted with 1 mL of ultrapure water and 5 mL of ethanol–*n*-hexane (60:50 v/v). The obtained solution was then sonicated and centrifuged for 15 min at 4000 rpm. The resulting pellet, after removal of the solvent phase by vacuum centrifugation, underwent two cycles of extraction–centrifugation under vacuum. A mixture of methyl *tert*-butyl ether and methanol (1:1 v/v) was added to the pellet and analyzed using the HPLC-DAD technique. The calibration curves were constructed using commercial standards of *β*-carotene and lutein purchased from Sigma-Aldrich (Milan, Italy). All analyses were performed in triplicate and the results were expressed as µg g^{-1} dw.

Determination of phenolic profile

For the detection and quantification of the microgreen polyphenol profile, an ultrahigh-performance liquid chromatography (UHPLC) system (Dionex Ultimate 3000, Thermo Fisher Scientific™, Waltham, MA, USA) coupled with a high-resolution mass spectrometry Orbitrap (Thermo Fisher Scientific™, Waltham, MA, USA) was used (supporting information, Table S1). Quercetin-3,4′-di-*O*-glucoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, rutin, myricetin, hyperoside, naringin, apigenin-7-*O*-glucoside, quercetin-glycuronide, isorhamnetin-3-*O*-galattoside and cyanidin-3-*O*-rhamnoside chloride (Extrasynthese, Lyon, France) were used as standards for quantitative and semi-quantitative analysis. Polyphenol chromatographic separation was carried out using a Luna Omega PS column (1.6 µm, 50 × 2.1 mm; Phenomenex, Torrance, CA, USA) thermostatically controlled at 25 °C. The mobile phase consisted of a biphasic solution: water (phase A) and acetonitrile (phase B), both containing 0.1% (v/v) formic acid. Polyphenolic compounds were eluted using the following gradient program: 0–1 min 0% B, 1–2 min 0–95% B, 2–2.5 min 95–95% B, 2.5–5 min 95–75% B, 5–6 min 75–60% B. Afterward, the gradient switched back to 0% B in 0.5 min and was held for 2.5 min for column re-equilibration. For all target compounds, an electrospray ionization source (Thermo Fisher Scientific™, Waltham, MA, USA) in negative ion mode was used, setting up two scanning events (full ion MS and full ion fragmentation, AIF). Full-scan data were acquired setting a resolving power of 35 000 FWHM (full width at half maximum) at *m/z* 200 whereas AIF scan events were acquired by setting a resolving power of 17 500 FWHM and collision energy values of 10, 20 and 45 eV. In both cases, the instrument was set to: spray voltage, −3.5 kV; sheath gas flow rate, 45 arbitrary units; auxiliary gas flow rate, 10 arbitrary units; capillary temperature, 275 °C; auxiliary gas heater temperature, 350 °C; S-lens RF level, 50; scan range, *m/z* 80–1250. Data processing was performed using Quan/Qual Browser Xcalibur software, v. 3.1.66.10 (Thermo Fisher Scientific™, Waltham, MA, USA). All analyses were performed in triplicate and the results were expressed as µg g^{-1} dw.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using IBM SPSS 28 software (International Business Machines Corporation, Armonk, NY, USA) and presented as mean ± standard error. One-way ANOVA was used to calculate the statistical significance of the mean effect of species (Sp) and priming treatment with

biostimulants (Bp). Two-way ANOVA was used to assess the significance of interaction effects between factors. The statistical significance of the Sp × Bp interaction and the Bp factor was determined using the Tukey–Kramer HSD test at a significance level of $P = 0.05$.

RESULTS

Biometric and yield parameters

Fresh yield (kg m^{-2}) and hypocotyl length (cm) shown in Fig. 1 were exclusively influenced by the effect of the species (Sp). Regardless of the priming treatment, Komatsuna exhibited the highest yield values (2.52 kg m^{-2}), while Mibuna had a longer hypocotyl length (4.92 cm).

Antioxidant capacity

The factors investigated and their interaction significantly influenced the antioxidant capacity, FRAP and ABTS (Table 1). Specifically, in terms of the antioxidant capacity of ABTS, Komatsuna did not show significant differences between the different priming treatments and the Control. On the contrary, for Mibuna, H_2O, A250 and H250 treatments increased ABTS antioxidant capacity by 22.25%, 35.67% and 54.73%, respectively, compared to Control. Regarding the antioxidant capacity of FRAP, H250 treatment recorded the highest values in both Mibuna and Komatsuna, with an increase of 22.42% and 47.93%, respectively, compared to Control.

Carotenoids

As presented in Table 2, β-carotene and lutein, determined by HPLC-DAD, were significantly influenced by the interaction of the two factors studied (Sp × Bp). For Komatsuna, the highest values of β-carotene were recorded in the Control

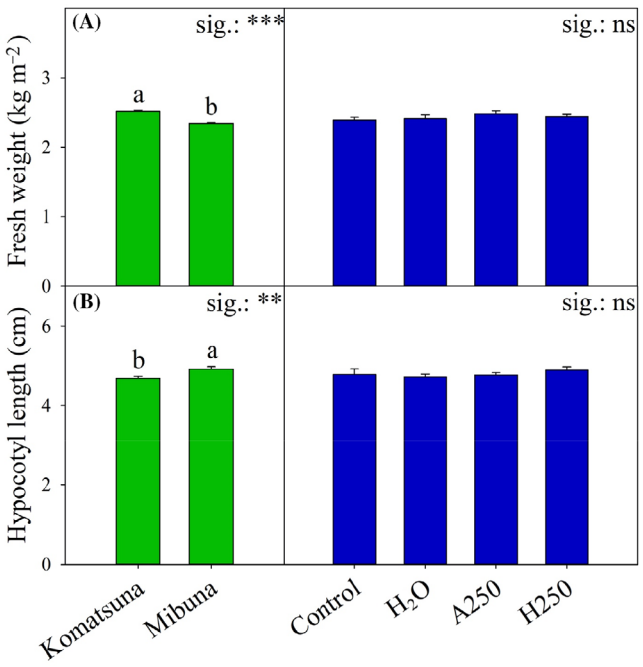

Figure 1. Effect of species and priming on fresh yield (A) and hypocotyl length (B). All data are expressed as mean ± standard error, $n = 3$. Different letters above bars indicate significant differences according to Tukey's HSD test. ns, non-significant; significant at $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$.

Table 1. Effect of species (Sp), biopriming treatments (Bp) and their interaction (Sp × Bp) on ABTS and FRAP antioxidant activities

Treatment	ABTS	FRAP
	mmol Trolox kg^{-1}	
Species (Sp)		
Komatsuna	45.85 ± 0.519	65.73 ± 2.908
Mibuna	102.24 ± 4.837	95.15 ± 2.131
Biopriming (Bp)		
Control	61.75 ± 8.095c	69.06 ± 7.560d
H_2O	71.17 ± 11.799b	77.37 ± 6.924c
A250	77.44 ± 13.776b	84.14 ± 5.668b
H250	85.81 ± 16.845a	91.20 ± 6.270a
Sp × Bp		
Komatsuna × Control	43.73 ± 0.218e	52.22 ± 0.674g
Komatsuna × H_2O	44.83 ± 0.114e	61.90 ± 0.269f
Komatsuna × A250	46.65 ± 0.118e	71.57 ± 1.424e
Komatsuna × H250	48.18 ± 0.216e	77.25 ± 1.266d
Mibuna × Control	79.77 ± 1.708d	85.89 ± 1.410c
Mibuna × H_2O	97.52 ± 1.412c	92.84 ± 0.513b
Mibuna × A250	108.23 ± 0.886b	96.71 ± 0.742b
Mibuna × H250	123.43 ± 1.745a	105.15 ± 0.590a
Significance		
Sp	***	***
Bp	***	***
Sp × Bp	***	**

All data are expressed as mean ± standard error, $n = 3$. Significant at $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$. Different letters within each column indicate significant differences according to Tukey's HSD test ($P = 0.05$).

Table 2. Effect of species (Sp), priming treatments (Bp) and their interaction (Sp × P) on carotenoid concentrations

Treatment	β-Carotene	Lutein
	μg g^{-1} dw	
Species (Sp)		
Komatsuna	1095.23 ± 38.611	748.49 ± 22.499
Mibuna	847.68 ± 34.988	497.79 ± 13.950
biopriming (P)		
Control	1104.43 ± 39.304a	654.47 ± 45.585b
H_2O	817.38 ± 25.950c	627.92 ± 44.687c
A250	937.37 ± 95.122b	647.40 ± 94.371a
H250	1026.63 ± 61.735b	562.76 ± 40.309d
Sp × Bp		
Komatsuna × Control	1191.37 ± 2.237	755.83 ± 4.937b
Komatsuna × H_2O	875.05 ± 3.302d	727.66 ± 5.055b
Komatsuna × A250	1150.03 ± 3.999b	858.06 ± 11.797a
Komatsuna × H250	1164.49 ± 2.177ab	652.39 ± 9.135c
Mibuna × Control	1017.49 ± 12.688c	553.10 ± 9.556d
Mibuna × H_2O	759.72 ± 5.589e	528.17 ± 3.205d
Mibuna × A250	724.72 ± 1.316f	436.74 ± 3.724f
Mibuna × H250	888.77 ± 6.815d	473.13 ± 2.668e
Significance		
Sp	***	***
Bp	***	***
Sp × Bp	***	***

All data are expressed as mean ± standard error, $n = 3$. Significant at $^{***}P \leq 0.001$. Different letters within each column indicate significant differences according to Tukey's HSD test ($P = 0.05$).

(1191.37 μg g^{-1} dw) and with H250 (1164.49 μg g^{-1} dw). On the other hand, for Mibuna, all priming treatments reduced β-carotene compared to the Control. Regarding lutein concentration, A250 and H250 treatments in Komatsuna showed an increase of 13.52% and a decrease of 13.68%, respectively, compared to the control. In Mibuna, the highest lutein values were obtained in the Control (533.10 μg g^{-1} dw) and H_2O treatment (528.17 μg g^{-1} dw).

Mineral concentration

The mineral composition of the microgreens varied with priming treatments with biostimulants (Bp) and was influenced by Sp × Bp interaction, as presented in Table 3. Nitrates, phosphorus and potassium were significantly influenced by the Sp × Bp interaction. The highest nitrate values were recorded in Komatsuna (on average). For both species, no significant differences in nitrate were observed between the Control and priming treatments. A similar trend was observed for potassium and partially for phosphorus. Regarding phosphorus, the A250 and H250 priming treatments increased its concentration by 6% (on average), compared to the Control. As presented in Table 3, the concentration of magnesium was influenced by Sp and Bp effects, while sulfur was influenced only by the Sp effect. However, no significant differences were observed for calcium, either for the main effects of Sp and Bp or for their interaction. Both magnesium and sulfur showed the highest values in Komatsuna. Regardless of the species, the priming treatments increased the magnesium concentration compared to the Control.

Phenolic compound concentration

Using UHPLC, 12 derivatives of phenolic acid and 23 derivatives of flavonoids were determined and quantified (Fig. 2 and supporting information, Tables S2 and S3). As shown in Fig. 2, the concentration of 3-coumaroylquinic acid in Mibuna was not influenced by the priming treatments. In contrast, for Komatsuna, the highest values were recorded in the A250 and H250 treatments. The opposite trend was observed for kaempferol-3-sinapoylsophoroside-7-glucoside and quercetin-3-glucuronide (Fig. 2), where no significant variation was observed in Komatsuna for the A250 and H250 priming treatments. In Mibuna, the values of kaempferol-3-sinapoylsophoroside-7-glucoside and quercetin-3-glucuronide in the A250 and H250 microgreens were on average about 1.3 and 4 times higher than those of the Control, respectively. Compared to the Control, priming treatments increased the concentration of total phenolic acid derivatives in both Mibuna and Komatsuna, with the highest values obtained from Komatsuna × A250 and Komatsuna × H250. In both species, the H250 treatment increased total flavonoid derivatives and total phenolic acids (Fig. 2) compared to the control treatments.

DISCUSSION

In light of the impressive results obtained for the application of biostimulants in the horticultural sector, we emphasize the enormous potential of these products. Currently, most studies of microgreens have focused on the effects of different cultivation parameters, neglecting the capabilities of biostimulant products. The short crop cycle, typical of microgreens, could be a major limitation for topical applications (foliar or root application) of

Table 3. Effect of species (Sp), priming treatments (Bp) and their interaction (Sp × Bp) on mineral concentrations

	Nitrate	P	K	S	Ca	Mg
Treatment	mg kg^{-1} fw	g kg^{-1} dw				
Species (Sp)						
Komatsuna	821.94 ± 16.967	7.88 ± 0.070	27.63 ± 0.477	8.23 ± 0.113	9.41 ± 0.124	4.04 ± 0.074
Mibuna	570.77 ± 14.517	5.14 ± 0.092	22.10 ± 0.266	5.75 ± 0.090	9.34 ± 0.229	3.14 ± 0.081
Biopriming (Bp)						
Control	719.28 ± 57.692a	6.25 ± 0.695b	25.29 ± 1.843	6.90 ± 0.647	8.89 ± 0.309	3.24 ± 0.230b
H_2O	707.21 ± 80.759ab	6.55 ± 0.634ab	25.24 ± 1.383	7.09 ± 0.568	9.62 ± 0.255	3.71 ± 0.220a
A250	652.33 ± 43.474b	6.47 ± 0.548b	23.98 ± 0.959	6.92 ± 0.587	9.29 ± 0.114	3.62 ± 0.210a
H250	706.60 ± 50.337ab	6.78 ± 0.586a	24.94 ± 0.980	7.04 ± 0.473	9.71 ± 0.209	3.81 ± 0.181a
Sp × Bp						
Komatsuna × Control	845.45 ± 15.727ab	7.79 ± 0.144a	29.33 ± 0.609a	8.33 ± 0.234	9.35 ± 0.195	3.71 ± 0.150bc
Komatsuna × H_2O	882.93 ± 12.917a	7.96 ± 0.038a	28.18 ± 0.973ab	8.34 ± 0.201	9.76 ± 0.367	4.18 ± 0.109ab
Komatsuna × A250	744.04 ± 26.419b	7.68 ± 0.168a	25.95 ± 0.592b	8.20 ± 0.277	9.12 ± 0.087	4.08 ± 0.079ab
Komatsuna × H250	815.35 ± 5.359ab	8.08 ± 0.106a	27.04 ± 0.370ab	8.04 ± 0.287	9.41 ± 0.228	4.21 ± 0.033a
Mibuna × Control	593.11 ± 21.804c	4.70 ± 0.074c	21.25 ± 0.509c	5.48 ± 0.104	8.43 ± 0.481	2.76 ± 0.135e
Mibuna × H_2O	531.49 ± 39.565c	5.13 ± 0.102bc	22.31 ± 0.093c	5.85 ± 0.179	9.47 ± 0.411	3.24 ± 0.086de
Mibuna × A250	560.62 ± 18.481c	5.26 ± 0.110b	22.01 ± 0.610c	5.65 ± 0.137	9.45 ± 0.171	3.16 ± 0.048de
Mibuna × H250	597.86 ± 28.539c	5.47 ± 0.032b	22.84 ± 0.498c	6.03 ± 0.172	10.01 ± 0.280	3.41 ± 0.067cd
Significance						
Sp	***	***	***	***	n.s.	***
Bp	*	***	n.s.	n.s.	n.s.	***
Sp × Bp	*	*	**	n.s.	n.s.	n.s.

All data are expressed as mean ± standard error, $n = 3$. n.s., non-significant; significant at $^*P \le 0.05$, $^{**}P \le 0.01$ and $^{***}P \le 0.001$. Different letters within each column indicate significant differences according to Tukey's HSD test ($P = 0.05$).

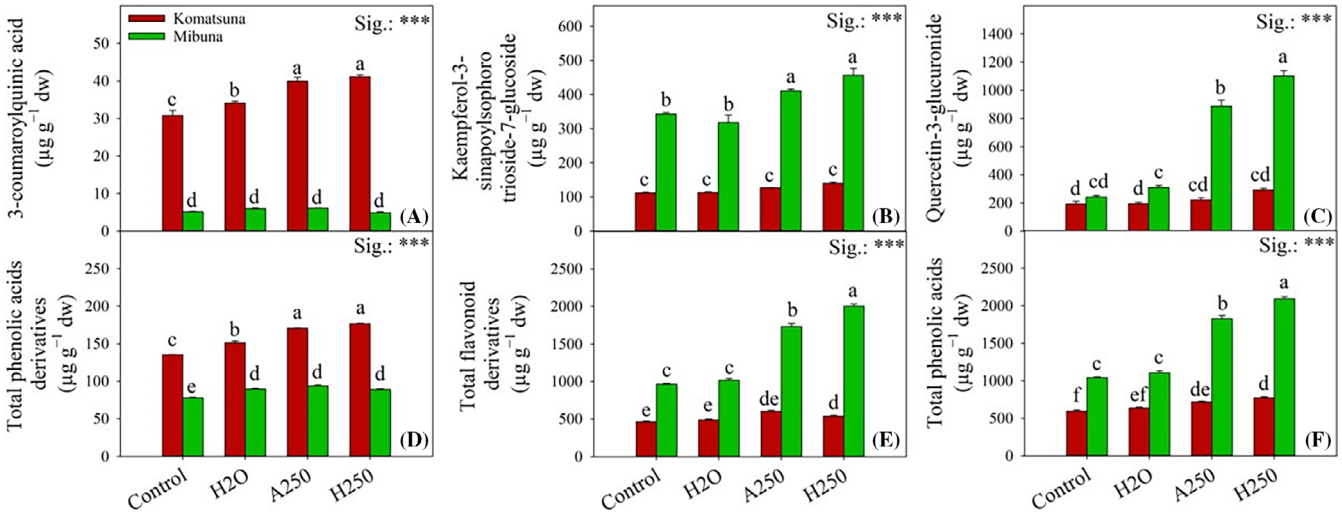

Figure 2. Effect of species × priming interaction on 3-coumaroylquinic acid (A), kaempferol-3-sinapoylsophorotrioside-7-glucoside (B), quercetin-3-glucuronide (C), total phenolic acid derivatives (D), total flavonoid derivatives (E) and total phenolic acids (F). All data are expressed as mean ± standard error, $n = 3$. Different letters above bars indicate significant mean differences according to Tukey's HSD test ($P = 0.05$).

biostimulant products. To overcome this problem, in this study, we evaluated the feasibility of applying biostimulants as priming agents to seeds of Brassicaceae (Mibuna and Komatsuna) harvested at the microgreen stage.

According to Ghoora *et al.*[32] and Kyriacou *et al.*,[33] the choice of genetic material is the primary factor that influences the fresh yield of microgreens. However, it is important to note that the differences in yield and yield parameters are not solely due to the phylogenetic distance between botanical families commonly used for microgreen production, as there is also intrafamily variability.[7] Our results confirm this, since Komatsuna showed higher production compared to Mibuna (Fig. 1(A)). Interestingly, the average fresh yield reported by Kyriacou *et al.*[33] for the same species, under similar experimental conditions (substrate, nutrition management and environmental conditions), was approximately 1.5 times higher than what we obtained in our experiment. These differences could be attributed to variations in the length of the crop cycle, as harvest timing significantly affects biomass production.[14] In our study, microgreens were harvested 14 DAS, while the experiment of Kyriacou *et al.*[33] had a duration of 16 days. Despite the higher biomass production in Komatsuna, it should be noted that Mibuna had longer hypocotyls. The lack of correlation between production and hypocotyl length may be due to a thinner hypocotyl and/or a lower distribution of leaf weight in Mibuna compared to Komatsuna. Despite previous bioassays showing that the application of plant-based biostimulants increases the hypocotyl length, dry weight of the root, biomass and leaf area[34,35] in seeds and young seedlings, our experiment did not observe any positive effect of priming with biostimulants on biometric parameters. As noted by Masondo *et al.*,[36] the positive effects of priming occur primarily when seeds are subjected to environmental stresses during the delicate pre- and post-germination phases, in addition to being influenced by seed size and treatment duration. The absence of stress conditions in our experiment may explain the lack of modification of the yield parameters observed in Komatsuna and Mibuna after priming treatments (Fig. 1(A),(B)). However, while the positive effects of biostimulants on growth and yield in mature plants are well debated and established,[35,37,38] there is currently limited information available for microgreens. Although El-Nakhel *et al.*[7] observed species-specific yield enhancement following the application of biostimulants via nutrient solution, a direct comparison with our results would not be scientifically valid due to differences in the mode of biostimulant application (priming *versus* integration in nutrient solution) and growth conditions (controlled parameter growth chamber *versus* passive-ventilated greenhouse). In addition, although the literature points out the positive effects of these products on yield performance, the mechanisms of action of these products are still being studied. Since this is one of the first studies on the application of priming with biostimulants for the production of microgreens, it is even more complex to provide a proper perspective on these results, considering that the positive action of biostimulants can be strongly influenced by the genetic material but also by the growth conditions.

Although increasing yield is the primary goal in microgreen cultivation, the growing interest in improving qualitative traits justifies the use of biostimulants and agronomic practices such as priming. Scientific evidence highlights that the importance of consuming microgreens in the diet is attributed to their more balanced and nutrient-rich mineral profile compared to their mature counterparts.[39,40] As previously described for yield parameters, genotype significantly influenced mineral concentration in microgreens, similar to observations by Xiao *et al.*[41] for 30 varieties of Brassicaceae. However, in partial agreement with de la Fuente *et al.*,[13] potassium was the most abundant mineral, followed by calcium, sulfur, phosphorus and magnesium (Table 3). Magnesium, a crucial mineral involved in more than 300 enzymatic reactions in the human body, immune system function and skeletal and muscular structure,[42] increased in both species with H250 priming treatment. In contrast, in Mibuna, the concentration of phosphorus, a fundamental nutrient for energy production, physiological response signaling and tissue maintenance,[43] increased in both the A250 and H250 priming treatments with biostimulants compared to the control.

Among the unique quality traits of leafy vegetables, nitrate, due to its antinutritional value, is undoubtedly the most deserving of attention, as it defines the safety of a final product. Although

microgreens fall into the category of leafy vegetables, data on nitrate concentration and the influence of different preharvest factors are still limited. However, similar to their mature counterparts,[44-46] nitrate levels in microgreens were significantly influenced by the species, with the highest values obtained from the most productive cultivar (Komatsuna; Table 3). As reported by Kyriacou *et al.*[33] and Niroula *et al.*,[47] this variability may depend on the different abilities of plant species to accumulate nitrates, as it is influenced by different metabolic activities. However, nitrate levels in Komatsuna were lower than those reported by Kyriacou *et al.*,[33] but similar to those recorded by Giordano *et al.*[48] for the same species. Although El-Nakhel *et al.*[7] observed an increase in the concentration of nitrate in dill after the application of a protein hydrolysate, in our study, regardless of the species, the priming treatment of A250 actually reduced the concentration of this antinutritional compound compared to the control. The explanation for this could be attributed to the regulation of nitrogen metabolism pathways by protein hydrolysates, since the chemical composition of these biostimulants (rich in amino acids) may have overloaded the phloem, reducing nitrate absorption and storage, or enhanced nitrate reductase activity.[49,50]

The interest in the production and consumption of microgreens is attributed to the richness and quality of phenolic compounds, as these are more complex compared to those of their mature counterparts.[4,51] The concentration and quality of the phenolic profile determine not only the sensory characteristics of microgreens but also their status as superfoods, as a diet based on the consumption of foods rich in these secondary metabolites may help prevent the onset of neurodegenerative and metabolic disorders.[5] As previously discussed for biometric parameters and mineral profiles, genotype played a crucial role in the biosynthesis of secondary metabolites, confirming the results of previous studies.[4,13,33,52] UHPLC analysis showed that total phenols concentration in Mibuna was 2.2 times higher than that of Komatsuna (Fig. 2(F)), despite the substantial qualitative similarities in the phenolic profile (supporting information, Tables S2 and S3). Consistent with the studies by Kyriacou *et al.*,[14] Tomas *et al.*[4] and Dereje *et al.*,[5] of the 35 identified phenolic compounds, the most represented chemical class was flavonoids (23 compounds), followed by phenolic acids (12 compounds). It should be noted that the concentration of quercetin-3-glucuronide (the most abundant flavonoid in Mibuna and Komatsuna) alone, regardless of the preparation treatment, exceeded the total concentration of all phenolic acids (Fig. 2(C),(D)). Considering that flavonoids and their derivatives (flavones and flavonols) have been shown to have higher bioavailability after gastrointestinal digestion compared to phenolic acids,[4] microgreens, due to their phenolic profile, are at the forefront of nutraceutical studies. The bioactivity of these secondary metabolites is attributed to their antioxidant capacity.[53] As previously reported by other authors,[17,22,53] our study observed a significant relationship between the concentration of total phenolic acids and antioxidant capacity (ABTS and FRAP). Specifically, regardless of priming treatments, Mibuna microgreens, characterized by a higher concentration of flavonoids and total phenolic compounds, had the highest ABTS (102.24 mmol Trolox kg^{-1}) and FRAP (95.15 mmol Trolox kg^{-1}) antioxidant capacity compared to Komatsuna, contrary to the findings of Kyriacou *et al.*[14] in a study of the same microgreen species. The different results may be attributed to the different growth conditions used and the different harvest stages. Consistent with this, the concentration of polyphenols is also influenced by preharvest factors other than genotype.[54] In our study, we observed a positive effect on the concentration of total phenolic acids for both microgreen species only when two biostimulants (A250 and H250) were used as preparation agents. Similarly, to the findings of El-Nakhel *et al.*,[7] the effects of biostimulant treatments were only related to the improvement of secondary metabolism. The explanation for this could be attributed to the presence of a large number of signaling molecules characteristic of biostimulants that induced the production of secondary metabolites. As suggested by Kisa *et al.*[55] and Feduraev *et al.*,[56] many of these bioactive molecules, in addition to serving as true precursors for secondary metabolites, directly act on the activation of key enzymes such as phenylalanine ammonia lyase and chorismate mutase. However, the efficacy of biostimulants in promoting the synthesis of polyphenols is not unique, as it varies with the type of biostimulant, plant species and type of bioactive compound.[35] To support this, it should be noted that the priming treatments (A250 and H250) promoted greater bioaccumulation of the main identified phenolic acid (3-coumaroylquinic acid; Fig. 2(A)) only in Komatsuna. On the contrary, for the flavonoids kaempferol-3-sinapoylsophoroside-7-glucoside and quercetin-3-glucuronide (Fig. 2(B),(C)), only Mibuna showed a positive effect of priming with biostimulants. These significant differences help in the understanding of the nonuniform response of ABTS and FRAP antioxidant capacity to priming treatments. Regarding the antioxidant capacity of FRAP, both in Komatsuna and Mibuna, an increase in antioxidant capacity was observed when priming with biostimulants was used, in line with the increase in total phenolic acids (Fig. 2(F) and supporting information, Table S2). However, for ABTS antioxidant capacity, the same trend was observed only in Mibuna. The different behavior of the antioxidant capacity could be attributed to substantial differences in the methodologies used which may not analyze all antioxidant compounds in the same way. Another explanation could lie in the fact that the relationship between antioxidant capacity and polyphenols is dependent not only on their concentration but also on their distribution throughout the entire profile. Since antioxidant capacity is a function of the chemical structure of each individual phenolic compound,[57] the increase in ABTS in Mibuna after priming treatments with biostimulants could be attributed solely to the increase in flavonoids. Another chemical class recognized for its antioxidant action is represented by carotenoids, hydrophobic bioactive molecules that play important physiological roles in the human body.[58] Contrary to the description of polyphenols, a higher concentration of β-carotene and lutein was recorded in Komatsuna. Furthermore, priming treatments with biostimulants did not have the same effects on the concentration of β-carotene and lutein concentration as observed for phenolic compounds. The explanation for this lies in the fact that the metabolic pathways of polyphenols and carotenoids are different.[59,60] In partial agreement with the findings of Xiao *et al.*[41] for 30 Brassicaceae species cultivated at the microgreen stage, both Mibuna and Komatsuna had β-carotene as the most abundant compound.

CONCLUSIONS

Microgreens have gained a significant market share among leafy vegetables due to their vibrant colors, bold flavors and premium nutraceutical properties, making them true superfoods. However, the growing interest in 'green' has necessitated the adoption of eco-friendly cultivation practices for microgreens as well, including the use of biostimulant products. In our work, the use of different priming products (protein hydrolysates and hydropriming) on

Komatsuna and Mibuna seeds did not result in significant differences in fresh yield and hypocotyl length but improved the qualitative traits. Overall, the priming treatments with biostimulants increased the concentration of antioxidant compounds such as total flavonoid derivatives and phenolic acids, consequently enhancing antioxidant capacity (ABTS and FRAP), thus contributing to increased nutraceutical value. In light of these promising results, future experiments should evaluate the response to priming with biostimulants in less productive microgreen species cultivated in suboptimal growing environments such as greenhouses or substrate-less cultivation systems with less concentrated nutrient solutions. The possibility of reducing the use of fertilizers, during the growth phases of microgreens, by taking advantage of the use of biostimulants could represent an interesting and new trend of research that would allow the production of microgreens to be enhanced even more not only as functional products for humans but also for the environment.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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