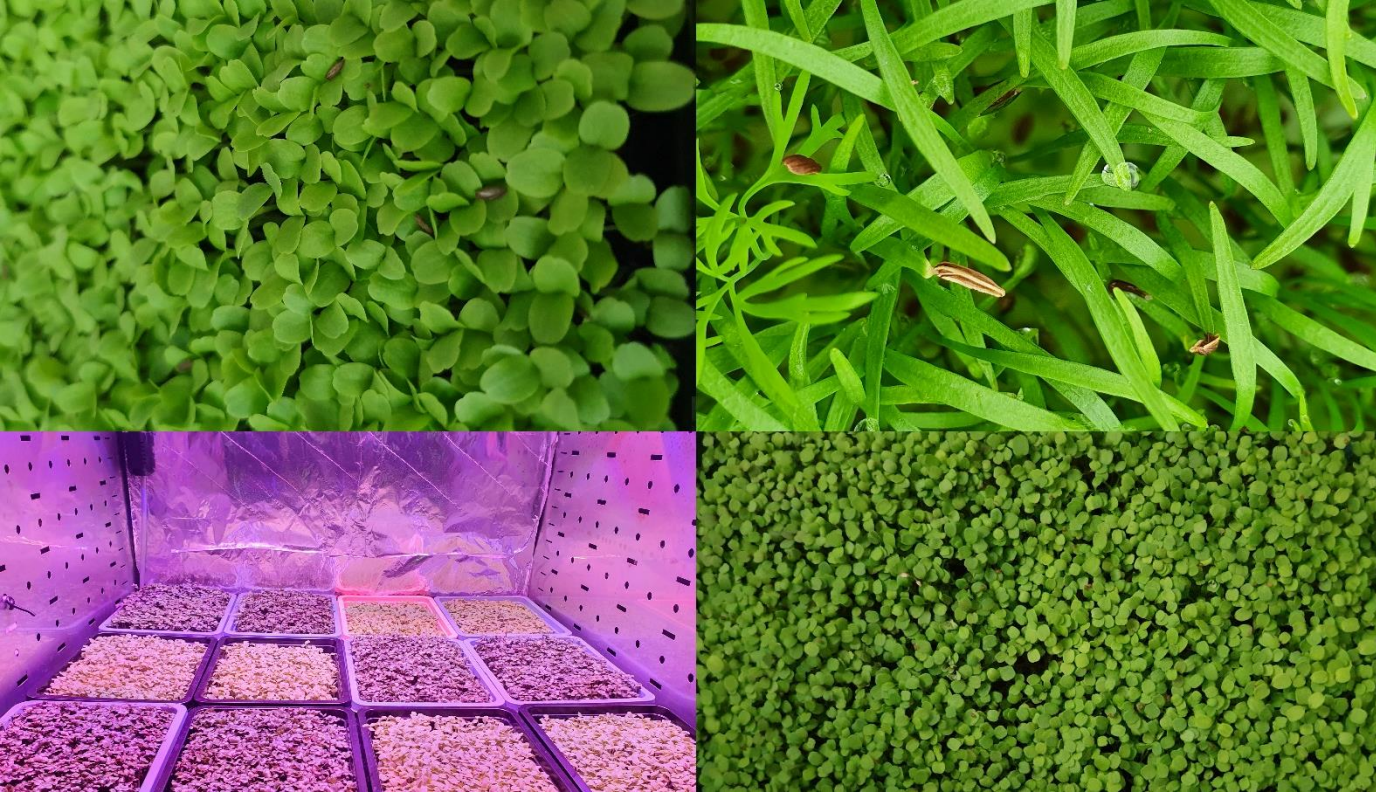

Unravelling postharvest quality in microgreens through modulation of preharvest factors

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Index

Publications

1. **Kyriacou, M.C.,** Antoniou, C., Rouphael, Y., Graziani, G., Kyratzis, A. **2021**. Mapping the primary and secondary metabolomes of carob (*Ceratonia siliqua* L.) fruit and its postharvest antioxidant potential at critical stages of ripening. ***Antioxidants***, 10, 57. https://doi.org/10.3390/antiox10010057.
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Chapter 1: General Introduction

Foods are fundamental in maintaining health and preventing chronic diseases by providing essential nutrients and calories for the body metabolism (Krehl, 1983). Based on clinical and pre-clinical trials, diets rich in vegetables provide an abundance of important secondary metabolites also known as phytochemicals such as ascorbic acid (vitamin C), tocopherols (vitamin E), glucosinolates, phenolic acids, anthocyanins, carotenoids and phylloquinone (vitamin K_1), which are known to prevent neuro-degenerative, cardiovascular, chronic (e.g., obesity) diseases and some forms of cancer (Rice-Evans and Miller, 1995). The new dietary guidelines (2015−2020) for Americans delivered from both the United States Department of Agriculture as well as the United States Department of Health and Human Services recommend 1−4 cups and 1-3 cups of vegetables per day for males and females, respectively, depending on their age (USDA, 2020). Over the past two decades, consumer and nutritionist interest in health promotion has been the driving force for the consumption of fresh, healthier (low calorific and fat content) and functional (phytochemicals-rich) foods (Xiao et al., 2012; Kyriacou et al., 2016; Choe et al., 2018).

Microgreens are a new class of specialty crop, defined as tender immature greens produced from the seeds of vegetables, herbs or grains, including wild species. The most popular microgreens are produced from seeds of the following plant families: *Asteraceae* (chicory, endive, lettuce, sunflower and radicchio), *Apiaceae* (carrot, chervil, parsley, carrot, celery, dill and fennel), *Amaryllidaceae* (garlic, leek, scallions, challots and onion), *Amaranthaceae* (amaranth, beet, quinoa, spinach and swiss chard), *Lamiaceae* (basil, chia and mint) and *Brassicaceae* (mustards, cabbages, broccoli, cauliflower, radishes, tatsoi, wasabi, arugula, cresses, kohlrabi,

mizuna, turnip, savoy, kale, komatsuna, pak choi, kogane, collard, nasturtium, brussel sprouts, rapini, rutabaga) (Choe et al., 2018; Turner et al., 2020). Microgreens are generally harvested at the soil level (2.5-7.5 cm in height), i.e., at the base of hypocotyls, upon appearance of the first and/or second true leaves, when cotyledons are fully expanded and still turgid, usually within 7-21 days from seed germination depending on the species/variety and growing conditions (Sun et al., 2013; Kyriacou et al., 2016). Microgreens are usually grown on both organic and inorganic substrates in controlled environments like growth modules, indoor and vertical farming as well as in commercial greenhouses (Galieni et al., 2020).

The idea of microgreens sprung from innovative chefs in the San Francisco bay, (Southern California, USA) where microgreens have been grown since the mid-90s. Initially, a few varieties (arugula, basil, beets, cilantro and kale) were offered. The microgreens market is growing year by year, with an annual growth rate of 8% during the forecasting period (2020-2028), especially in north America (Orsini and De Pascale, 2007) (www.mordorintelligence.com). As the COVID-19 pandemic has changed people's attitudes about their food shopping habits, do-it-yourself microgreens have offered a sustainable alternative. Moreover, in the last decade (2012-2021) the number of scientific publications (including both original research papers and reviews) on microgreens increased and the range of international journals hosting them was widened. Currently, more than 190 scientific articles are listed/indexed in the Scopus database (www.scopus.com) under the search term “microgreens”.

Microgreens gather an immense potential for adapting leafy vegetable production to a micro-scale, for improving nutritional value in the human diet and for influencing gastronomic trends. Their popularity stems from their vivid colors, delicate textures, unique flavor-enhancing properties as garnishes but also from their fortified phytonutrient content and potential bioactive value. Microgreens have much stronger flavor-enhancing properties than sprouts and usually contain higher amounts of phytonutrients and minerals and lower nitrates than their fully grown

counterparts (Xiao et al., 2012; Kyriacou et al., 2016). The interaction of genetic material with ecophysiological, pre- and postharvest conditions over nutraceutical and organoleptic characteristics of microgreens has recently attracted great interest among researchers and consumers. Accordingly, the proposed dissertation will examine how the modulation of preharvest factors impacts the quality and postharvest performance of microgreens.

Starting from the above considerations, the aim of the first experiment was to assess the genotypic variation underlying the phytochemical composition of microgreens. This can be a major contribution to this growing industry as the relative abundance of bioactive compounds across species and its implications for their sensory and functional quality may support future species selection. Accordingly, the first experiment provides an assessment of the variation encountered across 13 microgreens species/subspecies representing the botanical families *Brassicaceae*, *Chenopodiaceae*, *Lamiaceae*, *Malvaceae* and *Apiaceae*, which were grown under fully controlled growth chamber conditions. Moreover, information on plant secondary metabolite profiles and how these potentially bioactive compounds respond to LED spectral quality in new and emerging microgreens, like amaranth, cress, mizuna and purslane has been missing in the scientific literature. Therefore, a second experiment was carried out to understand the variation in productivity, mineral composition, antioxidant activity, target carotenoids, as well as qualitative/quantitative profiles of polyphenols in relation to the light spectra (red and blue LED light percentage composition). In fact, developing species-specific lighting systems to support all-around production and enhance important antioxidant compounds is affordable and imperative for the microgreens industry, which is characterized by high investment in technology - particularly lighting – driven by the necessity to cultivate new and highly fortified microgreen species in separate modules/growth chambers with a specific lighting systems. In this respect, works like the second experiment may contribute significantly to the pool of systematic information required to understand how the chemosphere of microgreens is

configured by the genotype-light-spectrum nexus and consequently how particular species may be streamlined for production under targeted optimal conditions. In addition, as the microgreens industry expands, so does the demand for a wider genotypic basis of the crop and more efforts are called to elucidate species-specific responses to light quality, particularly as regards functional quality and the synthesis of secondary metabolites in emerging genotypes as those reported for the first time in the second experiment.

Although several studies revealed that variation in microgreens' content of bioactive compounds is based on several pre-harvest factors such as genetic material (i.e., species; experiment 1), conditions of cultivation and light parameters (i.e., spectral quality; experiment 2), additional variables have also been implicated in shaping microgreens' nutritive and phytochemical composition, including the choice of growth medium. Therefore, a third experiment aimed to characterize and elucidate the modulatory effects of natural fiber substrates (agave fiber, coconut fiber and peat moss) and synthetic substrates (capillary mat and cellulose sponge) on the nutritional and phytochemical composition (minerals, nitrate, chlorophylls, target carotenoids, ascorbate and polyphenols) of select microgreens (coriander, kohlrabi and pak choi).

Another aspect was also addressed in the present dissertation, which concerns the definition of the optimal developmental stage for harvesting microgreens, which still remains fluid. The superior phytochemical content of microgreens against mature leaves postulated in previous works, has underpinned the current hypothesis that significant changes in compositional profile may take place during the brief interval of microgreens' ontogeny from the appearance of the first (S1) to the second true leaf (S2). Accordingly, in the fourth experiment microgreens of four brassicaceous genotypes (Komatsuna, Mibuna, Mizuna and Pak Choi) grown under controlled conditions and harvested at S1 and S2 were appraised for fresh and dry yield traits. They were further analyzed for macro- and micromineral content, carotenoid content, volatile organic compounds, anthocyanins and polyphenols, chlorophyll and ascorbate concentrations as well as *in vitro* antioxidant capacity.

Finally, three nitrate accumulating species (rocket, lettuce and mustard) were used for microgreens production in a controlled climate chamber and examined under nutrient deprivation eustress (experiment 5). Three different nutrient interruption methods (nutrient solution deprivation at 0, 6 or 12 days before harvest) were adopted during the growth cycle in order to modulate nitrate and mineral content as well as key secondary metabolites (phenolic acids and carotenoids) of these selected microgreen genotypes.

Chapter 2: Functional Quality in Novel Food Sources: Genotypic Variation in the Nutritive and Phytochemical Composition of Thirteen Microgreens Species

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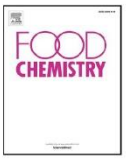

Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species

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Functional Quality in Novel Food Sources: Genotypic Variation in the Nutritive and Phytochemical Composition of Thirteen Microgreens Species

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Abstract

Compositional variation was examined across 13 microgreens species/subspecies representing *Brassicaceae*, *Chenopodiaceae*, *Lamiaceae*, *Malvaceae* and *Apiaceae*, grown in controlled environment. Macro-mineral concentrations were determined by ion chromatography, chlorophyll and ascorbate concentrations, and hydrophilic/lipophilic antioxidant potentials by spectrophotometry, and major carotenoids by HPLC-DAD. Nitrate hyper-accumulators and wide genotypic differences in Na, K and S concentrations were identified. Antioxidant capacity was highest in brassicaceous microgreens and significant genotypic variation was demonstrated in chlorophyll and carotenoid concentrations. High phenolic content was confirmed in *Lamiaceae* microgreens, with significant varietal differences, and alternative phenolics-rich microgreens from the *Apiaceae* were identified. Twenty-eight phenolic compounds were variably detected and quantitated through Orbitrap LC-MS/MS with flavonol glycosides, flavones and flavone glycosides, and hydroxycinnamic acids representing 67.6, 24.8 and 7.6% of the mean total phenolic content across species, respectively. The obtained information is critical for selecting new species/varieties of microgreens that may satisfy demand for both taste and health.

Keywords: *antioxidant activity, ascorbate, carotenoids, flavonoids, hydroxycinnamic acids, macro-minerals, phenolic compounds.*

Chemical compounds studied in this article:

Apigenin-7-O-glucoside (PubChem CID: 12304093)

Chlorogenic acid (PubChem CID: 1794427)

Ferulic acid (PubChem CID: 445858)

Kaempferol-7-O-glucoside (PubChem CID: 10095180)

Luteolin-7-O-glucoside (PubChem CID: 5280637)

Quercetin-3-O-galactoside (PubChem CID: 90657624)

Rosmarinic acid (PubChem CID: 5281792)

Rutin (PubChem CID: 5280805)

Vitexin (PubChem CID: 5280441)

1. Introduction

Microgreens constitute a novel specialty crop, defined as immature greens harvested without roots from the tender seedlings of vegetables, herbs, grains and wild crop relatives (Xiao et al., 2012; Kyriacou et al., 2016a). Aside from being a gastronomic novelty, often referred to as *vegetable confetti*, microgreens constitute an emerging class of fresh functional foods with an immense potential for enhancing human diet and addressing nutritional deficiencies due to their potent phytochemical content and their adaptability to large-scale commercial as well as localized small-scale or even domestic production (Kyriacou et al., 2016a). The nutritive and functional value of micronutrients for human health rests in their rich phytonutrient content that includes mineral macro and microelements (Ca, Mg, Fe, Mn, Zn, Se and Mo) (Pinto et al., 2015), carotenoids (β-carotene, lutein/zeaxanthin, neoxanthin, violaxanthin) (Xiao et al., 2012; Brazaitytė et al., 2015; Samuolienė et al., 2017) ascorbic acid (vitamin C), α- and γ-tocopherols (vitamin E group) and phylloquinone (vitamin K1) (Xiao et al., 2012). Additionally, the young tissues of microgreens represent potentially rich sources of phenolic compounds. These constitute one of the largest categories of secondary metabolites in plants, characterized by the presence of phenol rings and further subdivided according to the nature of substitutions on these aromatic rings into classes such as the flavonoids (e.g., flavones, isoflavones, anthocyanins), the phenolic acids (hydroxylbenzoic and hydroxycinnamic acids), stilbenes, lignans, lignins and tannins (Balasundram et al., 2006). Plants are preeminently abundant sources of flavonoids which are low molecular weight phenolic compounds consisting of two aromatic rings usually joined by a heterocyclic 3-carbon ring, the substitutions patterns to which differentiate flavonoids into flavonols, flavones, flavanones, flavanols, isoflavones, flavanonols, and anthocyanidins (Hollman and Katan, 1999). Phenolic compounds have important roles in plant reproduction (e.g., flower and fruit coloration) and in plant chemodefense owing to their deterring taste to predators, while inadvertently they influence also the sensory profile of fruits and vegetables. Major health benefits against cardiovascular and degenerative ailments derived from systematic consumption of fresh fruits and vegetables have been associated largely with the antioxidant function of phenolic compounds (Balasundram et al., 2006).

As an emerging food source enjoying limited scientific information, the antioxidant potential, the phenolic profile and the overall phenolic content of microgreens have yet been scarcely examined. Using ultrahigh-performance liquid chromatography photodiode array high-resolution multistage mass spectrometry Sun et al., (2013) analyzed the phenolic composition of five *Brassicacea* microgreens species and have putatively identified a total of 164 polyphenols, including 105 flavonol glycosides and 29 phenolic acid derivatives, based on retention times, elution orders, UV−vis and high-resolution mass spectra, an in-house database and literature comparisons. They concluded that brassicaceous microgreens can be considered as good sources of food polyphenols. The total concentration of phenolics (expressed in gallic acid equivalents) in microgreens representing the *Amaranthaceae*, *Brassicaceae*, *Chenopodiaceae* and *Lamiaceae* families ranged 1.5-7.0 g kg^{-1} dw according to Xiao et al. (2015) and 164-328 mg g^{-1} fw according to Bulgari et al. (2017), with the highest concentration encountered in *Lamiaceae* and *Brassicaceae*.

Furthering our understanding of the genotypic variation in phytochemical composition of microgreens can be a major contribution to this growing industry, as the relative abundance of bioactive compounds across species and its implications for their sensory and functional quality may support future species selection. Accordingly, the current work provides an assessment of the variation encountered across 13 microgreens species/subspecies representing the botanical families *Brassicaceae*, *Chenopodiaceae*, *Lamiaceae*, *Malvaceae* and *Apiaceae*, which were grown under fully controlled temperature, humidity, nutrient supply, photoperiod, light intensity and light quality conditions. Analytical characterization encompassed the concentrations of minerals (NO_3^-, P, K, S, Ca, Mg, Na) determined by ion chromatography, the photometric determination of chlorophyll and ascorbate concentrations and the hydrophilic and lipophilic antioxidant potentials of microgreens, the separation and quantification of major carotenoids (β-carotene and lutein) by HPLC-DAD and the qualitative and quantitative profiles of polyphenols obtained using an Ultra High Pressure Liquid Chromatograph coupled to mass spectrometry Q Exactive Orbitrap LC-MS/MS. The obtained information on polyphenol profiles and on the overall compositional constitution of microgreens was subjected to Principal Component Analysis to appraise possible clustering patterns underlined by taxonomic affiliation and to determine which quality traits were the most effective in discriminating between microgreens species.

2. Materials and methods

2.1. Chemicals and standards

Phenolic and carotenoid standards were obtained as follows: chicoric acid, chlorogenic acid, caffeic acid, catechin, epicatechin, rosmarinic acid, ferulic acid, rutin, vitexin, quercetin-3-O-glucoside, lutein and *β*-carotene from Sigma (St. Louis, MO, USA); and quercetin-3-O-galattoside, kaempferol-7-*O*-glucoside, kaempferol-3-*O*-rutinoside, 3,5-Di-O-caffeoyl quinic acid from Extrasynthese (Genay, France). Methanol, and formic acid (LC–MS grade), were purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q Gradient A10 water purification system.

The purity of the standards was 98%, and all were prepared as initial stock solutions of 1 mg mL^{-1} in methanol. Lutein and *β*-carotene stocks of 1 mg mL^{-1} were prepared in chloroform. Multiple standards stock solutions were prepared by combining individual standard stock solutions and further dilutions with methanol were made to obtain standard calibration curves in the range of 0.01–5 mg L^{-1}.

2.2. Plant material and growth conditions

Thirteen microgreens species belonging to the families of *Apiaceae* (coriander), *Brassicaceae* (cress, kohlrabi, komatsuna, mibuna, mustard, pak choi, radish, tatsoi), *Lamiaceae* (green and purple basil), *Malvaceae* (jute), and *Chenopodiaceae* (Swiss chard) were evaluated for their phytochemical composition. Common and scientific nomenclature, botanical family, cultivar, seed source, 100-seed weight, sowing density as well as the growth cycle length of the microgreens are reported in **Supplementary Table S1**.

All microgreens samples were obtained from controlled culture performed in growth chamber environment (KBP-6395F, Termaks, Bergen, Norway) at the Department of Vegetable Crops of the Agricultural Research Institute (ARI), Nicosia, Cyprus. The thirteen microgreens species were grown in plastic trays (14 × 19× 6 cm) containing commercial peat-based substrate of pH 6.3 and electrical conductivity (EC) of 0.2 dS m^{-1}, porosity 92% v/v, cation exchange capacity 98 meq 100 g^{-1}. Microgreens were fertigated with a quarter-strength Hoagland and Arnon formulation prepared using deionized water and containing: 2.0 mM NO_3-N, 0.25 mM S, 0.20 mM P, 0.62 mM K, 0.75 mM Ca, 0.17 mM Mg, 0.25 mM

NH_4-N, 20 µM Fe, 9 µM Mn, 0.3 µM Cu, 1.6 µM Zn, 20 µM B, and 0.3 µM Mo yielding an EC of 0.3 dS m^{-1} and a pH of 6.0.

Day/night temperatures of 22/18 ± 2°C were established with a 12 h photoperiod and a relative air humidity of 65-75%. Microgreens were grown under Light Emitting Diode (LED) panel units (K5 Series XL750, Kind LED, Santa Rosa, CA, USA) with an emission wavelength ranging 400-700 nm divided into three customizable channels: red (600-700 nm), blue (400-500 nm) and green-yellow (500-600 nm). The LED panel was arranged in the growth chamber so as to ensure a homogeneous photosynthetic photon flux density (PPFD) of 300 ± 10 µmol m^{-2} s^{-1} over the entire surface of the shelf (approx. 0.4 m^2). The PPFD and the spectral composition were measured and regulated at the substrate level using a spectral radiometer (MSC15, Gigahertz-Optik, Türkenfeld, Germany) in order to match the radiation spectrum to the optimal absorption spectrum of photosynthesis. The substrate trays were arranged randomly and systematically rotated every 24 h to enhance the uniformity of the light environment. The entire experiment was replicated three time.

Supplementary Table 1. Common names, botanical nomenclature, cultivar names, seed sources, 100-seed weight, sowing density and duration of growth period for 13 microgreens species/subspecies grown under controlled environment and assessed for nutritive and phytochemical composition.

Common name	Scientific name	Family	Cultivar	Source	100-seed weight (g)	Sowing density (seeds m^{-2})	Growth period (days)
Coriander	*Coriandrum sativum* L.	*Apiaceae*	Micro Splits	CN Seeds Ltd, Pymoor, Ely, Cambrigeshire, UK	0.684	46000	20
Cress	*Lepidium sativum* L.	*Brassicaceae*	Curled	CN Seeds Ltd, Pymoor, Ely, Cambrigeshire, UK	0.273	60000	16
Green basil	*Ocimum basilicum* L.	*Lamiaceae*	-	Orto del Sole, Bari, Puglia, Italy	0.137	70000	20
Jute	*Corchorus olitorius* L.	*Malvaceae*	Syrian	Nehme Establishment For Trade & Agriculture, Batroun, Lebanon	0.160	63700	22
Kohlrabi	*Brassica oleracea* L. var. *gongylodes*	*Brassicaceae*	Purple Vienna	Condor Seed Production, Yuma, Arizona, USA	0.320	60000	22
Komatsuna	*Brassica rapa* L. var. *perviridis*	*Brassicaceae*	Comred F1	CN Seeds Ltd, Pymoor, Ely, Cambrigeshire, UK	0.257	56700	16
Mibuna	*Brassica rapa* L. subsp. *nipposinica*	*Brassicaceae*	Japanese greens	CN Seeds Ltd, Pymoor, Ely, Cambrigeshire, UK	0.266	68600	16
Mustard	*Brassica juncea* (L.) Czern.	*Brassicaceae*	Osaka purple	Condor Seed Production, Yuma Arizona, USA	0.190	60000	20
Pak choi	*Brassica rapa* L. subsp. *chinensis*	*Brassicaceae*	Red Wizard F1	CN Seeds Ltd, Pymoor, Ely, Cambrigeshire, UK	0.240	63000	16
Purple basil	*Ocimum basilicum* L.	*Lamiaceae*	-	Condor Seed Production, Yuma, Arizona, USA	0.165	70000	19
Radish	*Raphanus sativus* L.	*Brassicaceae*	Sango	Condor Seed Production, Yuma, Arizona, USA	1.313	51000	22
Swiss Chard	*Beta vulgaris* L. subsp. *vulgaris*	*Chenopodiaceae*	Golden Sunrise	Condor Seed Production, Yuma, Arizona, USA	1.260	40000	22
Tatsoi	*Brassica rapa* L. subsp. *narinosa*	*Brassicaceae*	-	Condor Seed Production, Yuma, Arizona, USA	0.220	70000	16

2.3. Collection of microgreens samples

At the appearance of the first true leaves, microgreens in each plastic tray were harvested just above the substrate level using sterilized scissors. Fresh yield was expressed in kg m^{-2}. For compositional and phytochemical analyses, samples of fresh matter from randomly selected microgreens were instantly frozen in liquid nitrogen and stored at -80 ºC before lyophilized in a Christ, Alpha 1-4 (Osterode, Germany) freeze drier. Determination of yield, dry matter, colorimetry, ascorbate and pigment concentrations were performed at the ARI, whereas nitrate content, antioxidant activity, mineral composition, and phenolic profiles were determined on desiccated and lyophilized samples dispatched to the University of Naples Federico II, Italy.

2.4. Microgreens colorimetric assessment

Just before harvesting, microgreens canopy color was measured at three different points of each plastic tray using an 8 mm-aperture Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd, Osaka, Japan) calibrated with a Minolta standard white plate before measurements were performed. Measurements were obtained in the *Commission internationale de l'éclairage* CIELAB colour space parameters: L* (lightness, ranging from 0 = black to 100 = white), a^* [chroma component ranging from green (-60) to red (+60)], b^* [chroma component ranging from blue (-60) to yellow (+60)]. From the recorded parameters a* and b* the chroma (C*), which denotes the overall color intensity (chromaticity), was calculated using the following formula $(a^2 + b^2)^{1/2}$.

2.5. Dry matter, nitrate and mineral content analysis

The microgreens dry matter content was determined in triplicates following the official method 934.01 of the Association of Official Analytical Chemists. Briefly, the dry matter was calculated as a percentage of microgreens fresh mass (stem and leaves) following desiccation in a forced-air oven at 65 °C to constant weight (around 72 h) as determined on an analytical balance (Denver Instruments, Denver, Colorado, USA).

Desiccated microgreens (stem and leaves) were ground in a Wiley Mill to pass through an 841-microns screen, and then samples of the dried tissues were used for chemical analyses. Microgreens were assayed for concentrations of NO_3-N and the following macroelements: P, K, S, Ca, Mg and Na. Briefly, 0.2 g of finely ground dried tissues were

suspended in 50 ml of ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany) and subjected to three freeze-thaw cycles in liquid nitrogen followed by shaking water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 ºC for 10 min. The mixture was centrifuged at 6000 rpm for 10 min (R-10M, Remi Elektrotechnik Limited, India), then filtered through a 0.20 µm filter paper (Whatman International Ltd., Maidstone, U.K.). Potassium, Ca, Mg and Na were separated by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) and quantified through an electrical conductivity detector. Chromatographic separation was achieved in isocratic mode on an IonPac CS12A analytical column (4×250 mm, Dionex, Corporation) equipped with an IonPac CG12A precolumn (4×250 mm, Dionex, Corporation) and a self-regenerating suppressor CERS500 (4 mm, Dionex, Corporation). The NO_3^-, P and S contents were also quantified through ion chromatography coupled to a conductivity detector. An IonPac ATC-HC anion trap (9×75 mm), and an AS11-HC analytical column (4×250 mm) equipped with an AG11-HC precolumn (4×50 mm) and a self-regenerating suppressor AERS500 (4 mm) were used for separation. Nitrate was expressed as mg kg^{-1} fresh weight (fw) on the basis of each sample's original dry weight content (dw), while P, K, S, Ca, Mg and Na were expressed as g kg^{-1} dw.

2.6. Photosynthetic pigments, ascorbic acid and antioxidant activities

For the extraction of photosynthetic pigment chlorophyll, 200 mg of microgreens fresh weight were extracted by grinding the stem and leaves in ammoniacal acetone using mortar and pestle. The resulting extracts were centrifuged at 3000g for 3 min. The extracts' chlorophyll content was determined based on the absorbance at 647 and 664 nm using a Hach DR 2000 spectrophotometer (Hach Co., Loveland, Colorado, USA). Formulae and extinction coefficients used for the determination of chlorophyll *a*, *b* and total chlorophyll were described in detail by Lichtenthaler and Wellburn (1983). Chlorophyll analyses were performed in triplicate and the content of chlorophyll *a*, *b* and total chlorophyll were expressed in mg kg^{-1} of microgreens' fresh weight.

The total ascorbic acid, defined as the sum of ascorbic and dehydroascorbic acids, was assessed by spectrophotometric assays based on the reduction of Fe^{3+} to Fe^{2+} by ascorbic acid and the spectrophotometric detection of Fe^{2+} complexes with 2,2-dipyridyl (Kampfenkel et al., 1995). Dehydroascorbate was first reduced to ascorbic acid by pre-incubation of the sample in dithiothreitol. Quantitation was performed at 525 nm against an

external ascorbate standard calibration curve in the range of 5-100 μmol mL^{-1} and the results were expressed as mg kg^{-1} fw.

For assessing the antioxidant capacity of microgreens, 200 mg of lyophilized material underwent two different extraction procedures. The hydrophilic fraction was extracted with distilled water and its antioxidant activity was measured with the N,N-dimethyl-p-phenylenediamine (DMPD) method (Fogliano et al., 1999). The lipophilic fraction was extracted with methanol and the antioxidant activity of this extract was measured with the 2,20-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS method (Pellegrini et al., 1998). Both hydrophilic and lipophilic antioxidant activities were determined by UV–Vis spectrophotometry. For the hydrophilic antioxidant activity, an ascorbate external standard calibration curve was prepared in the range of 0.1-1.0 mg mL^{-1} based on a minimum of six concentration levels and determined by regression coefficients R^2>0.99. For the lipophilic antioxidant activity, a Trolox external standard calibration curve was prepared in the range of 2-20 μmol mL^{-1} based on a minimum of six concentration levels and determined by regression coefficients R^2>0.99. The absorbance of the solutions was measured at 505 and 734 nm, respectively. Hydrophilic and lipophilic fractions were expressed as mmol ascorbic acid and Trolox (6-hydroxy-2,5,7,8-tetramethylchro man-2-carboxylic acid) per kg dw, respectively.

2.7. Extraction and preparation for polyphenol assays

One hundred mg of lyophilized microgreens were extracted with 5.0 mL of methanol/water (60:40, *v/v*) by sonication at room temperature for 30 min. The mixtures were centrifuged at 4000 rpm for 15 min at room temperature, filtered through a 0.45 μm filter paper (Whatman International Ltd., Maidstone, U.K.), and then 10 μL of the extract was used for mass spectrometry (HRMS-Orbitrap) analysis.

2.8. UHPLC-Q-Orbitrap HRMS analysis

Qualitative and quantitative profiles of polyphenols were obtained using an Ultra High Pressure Liquid Chromatograph (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a degassing system, a Dionex Ultimate 3000 Quaternary UHPLC pump working at 1250 bar, an auto sampler device and a thermostated (T = 25°C) Kinetex 1.7 μm Biphenyl (100 × 2.1 mm) column (Phenomenex, Torrance, CA, USA). Injection volume was

of 2 µL. Gradient elution was built with two mobile phases: phase A (0.1% formic acid in H_2O), phase B (0.1% formic acid in methanol). All metabolites were eluted using a 0.2 mL min^{-1} flow rate with a gradient programmed as follows: 0 min - 5% of phase B, 1.3 min - 30% of phase B, 9.3 min - 100% of phase B, 11.3 min - 100% of phase B, 13.3 min - 5% of phase B, 20 min - 5% of phase B.

For the mass spectrometry analysis, a Q Exactive Orbitrap LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA) was used. An ESI source (HESI II, Thermo Fischer Scientific, Waltham, MA, USA) operating in negative ion mode (ESI-) for all the analyzed compounds was used. Ion source parameters in (ESI-) mode were: spray voltage -2,8 kV, sheath gas (N2>95%) 45, auxiliary gas (N2>95%) 10, capillary temperature 275 °C, S-lens RF level 50, auxiliary gas heater temperature 305 °C. The targeted acquisition of the polyphenolic compounds was carried out on parallel reaction monitoring (PRM) mode, with the following instrument settings: microscans at 1, resolution at 35,000, AGC target at 5e5, maximum ion time at 100 ms, MSX count at 1, isolation window at 1.0 m/z. For precise detection, the polyphenolic compounds were added to an inclusion list with input time frame for elution and collision energy (CE) optimized for each compound. The accuracy and calibration of the Q Exactive Orbitrap LC-MS/MS was checked daily using a reference standard mixture obtained from Thermo Fisher Scientific. The mass tolerance window was set to 5 ppm for the two analysis modes. The linearity of the method was assessed at both low (LOQ – 5 mg kg^{-1}) and high (5 mg kg^{-1} – 120 mg kg^{-1}) concentration ranges, using six concentration levels in each calibration range. Sensitivity was evaluated by limits of detection (LODs) and limits of quantitation (LOQs). Data analysis and processing were performed using the Xcalibur software, v. 3.0.63 (Xcalibur, Thermo Fisher Scientific). UHPLC-HRMS parameters for the identification of phenolic compounds of 13 microgreens species/subspecies grown under controlled environment are presented in **Supplementary Table 2**.

Supplementary Table 2. UHPLC-HRMS parameters for the identification of phenolic compouds of 13 microgreens species/subspecies grown under controlled environment.

ID peak	Compound	Molecular formula	RT (min)	Theoretical $[M-H]^-$	Measured $[M-H]^-$	Mass accuracy (ppm)	Fragment
28	1,2-disinapoyl-2-feruloylgentiobiose	$C_{44}H_{50}O_{22}$	12.04	929.37210	929.35278	-20.788	393.18948
24	apigenin-7-O-glucoside	$C_{21}H_{20}O_{10}$	10.58	431.09837	431.09845	0.186	269.04581
6	caffeic acid	$C_9H_8O_4$	8.41	179.03498	179.03485	-0.726	135.04433
2	caffeic acid hexoside isomer	$C_{15}H_{18}O_9$	7.79	341.088810	341.09	3.489	178.03487
8	Cirsilol	$C_{17}H_{14}O_7$	8.92	329.066680	329.06775	3.252	269.04666
4	chlorogenic acid	$C_{16}H_{17}O_9$	8.29	353.088810	353.08983	2.889	191.05598
13	Coumaric acid	$C_9H_8O_3$	9.73	163.040070	163.0403	1.411	119.05028
18	dicaffeoyl-quinic acid isomer	$C_{25}H_{24}O_{12}$	10.00	515.119500	515.1193	-0.388	353.08752
20	ferulic acid	$C_{10}H_{10}O4$	10.05	193.050630	193.0506	-0.155	178.02721
11	feruloylquinic acid isomer	$C_{17}H_{20}O_9$	9.56	367.103460	367.10364	0.490	191.05606
19	isorhamnetin-3-O-gentobioside	$C_{28}H_{32}O_{17}$	10.05	639.156670	639.15692	0.391	315.05102
5	kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside	$C_{42}H_{64}O_{24}$	8.39	933.230630	933.23218	1.661	771.17761
14	kaempferol-3-O-(coumaroyl)-sophoroside-7-O-glucoside	$C_{42}H_{46}O_{23}$	9.79	917.235710	917.23706	1.472	755.18292
9	kaempferol-3-O-(synapoyl)-sophoroside-7-O-glucoside	$C_{44}H_{50}O_{25}$	8.95	977.256840	977.25818	1.371	815.20508
27	kaempherol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	11.64	593.151190	593.152270	1.821	285.04068
26	kaempferol-7-O-glucoside	$C_{21}H_{20}O_{11}$	11.09	447.093280	447.09341	0.291	285.04059
22	luteolin-7-O-rutinoside	$C_{27}H_{30}O_{15}$	10.38	593.151190	593.15163	0.742	285.04074
25	luteolin-malonil-hexose	$C_{24}H_{22}O_{14}$	10.84	533.083680	533.0781	-10.467	285.04004
7	luteolin-7-O- glucoside	$C_{21}H_{20}O_{11}$	8.84	447.093290	447.08646	-15.276	285.04062
12	quercetin-3-O-galattoside	$C_{21}H_{20}O_{12}$	9.69	463.08819	463.08841	0.475	301.03555
23	quercetin-3-O-glucoside	$C_{21}H_{20}O_{12}$	10.53	463.08819	463.08972	3.304	301.03568
3	quercetin-sophoroside	$C_{27}H_{30}O_{17}$	8.24	625.14302	625.14417	1.840	462.08087
17	quercetin-3-O-(feruloyl)-sophoroside-7-O-glucoside	$C_{43}H_{48}O_{25}$	9.92	963.24119	963.24243	1.288	801.18927
10	quercetin-3-O-sophoroside-7-O-glucoside	$C_{33}H_{40}O_{22}$	9.25	787.19384	787.19189	-2.477	301.03561
21	quercetin malonyl glucoside	$C_{24}H_{20}O_{15}$	10.28	549.18859	549.17218	-29.880	301.02765
16	rosmarinic acid	$C_{18}H_{16}O_8$	9.90	359.07724	359.07018	-19.662	197.08206
15	rutin	$C_{27}H_{30}O_{16}$	9.89	609.14611	609.14642	0.509	301.03516
1	vitexin	$C_21H_{20}O_{10}$	6.49	431.09937	431.09808	-2.992	311.05661

2.9. Extraction and preparation for carotenoid assays

The procedure used for the extraction of carotenoids is a modification of the method described by (Kim et al., 2008). The dried sample powder (100 mg) was mixed with 6 mL of ethanol containing 0.1% BHT. The samples were placed in an 85 °C water bath for 5 min, after which 120 μL of 80% KOH was added to each mixture. Samples were then vortexed and returned to the water bath for 10 min for saponification. The reacted solution was then immediately placed in ice and 3 mL of distilled water and 3 mL of hexane were added to the solution. After centrifugation, the hexane layer containing the crude carotenoids was collected and the pellet was re-extracted twice more using hexane. The hexane layers were combined and completely dried with nitrogen gas. The residue was recovered with 1 mL of chloroform, filtered through a 0.2 μm nylon filter and immediately analyzed by HPLC.

2.10. Quantification of carotenoids by HPLC-DAD

Reverse Phase–HPLC separation was carried out using a Shimadzu HPLC Model LC 10 (Shimadzu, Osaka, Japan) and a 250×4.6 mm, 5 μm Gemini C18 column (Phenomenex, Torrance, CA, USA). Twenty microliters of each sample were injected and eluted with acetonitrile as mobile phase A and ethanol:*n*-hexane:dichloromethane (1:1:1) as phase B at a flow rate of 1 mL min^{-1}. A gradient of mobile phases A and B was built over a 25 min run as follows: 0–8 min (82:18); 8-12 min (76:24); 12-18 min (39:61); and 18-25 min a linear gradient from 39:61 to 82:18 for equilibration. The absorbance of the eluent was measured at 450 nm. Authentic lutein and *β*-carotene were used to evaluate their quantity in the sample based on external calibration curves ranging 5 to 100 μg mL^{-1} including a minimum of six levels of concentration.

2.11. Statistics

Analysis of variance (one way-ANOVA) of the experimental data was performed using the SPSS 20 software package. To separate treatment means within each measured parameter, the Duncan's Multiple Range Test was performed at $P \leq 0.05$. Yield, color, mineral composition and bioactive phytochemical composition of microgreens were subjected to Principal Component Analysis (PCA) to explore relationships among variables and treatments and to determine which quality traits were the most effective in discriminating between microgreens species. The PCA outputs include variable loading to

each selected component and treatment component scores. Principal component analysis was carried out using function 'PCA' from the SPSS 20 software package.

3 Results and discussion

3.*1. Growth, yield and colorimetric characteristics*

The time from sowing to harvesting mature microgreens at the second true leaf stage differed considerably between the thirteen species examined. Five species (cress, komatsuna, mibuna, pak choi and tatsoi) were harvested 16 days after sowing, one species (purple basil) at 19 days, three species (coriander, green basil and mustard) at 20 days, and four species (jute, kohlrabi, radish and Swiss chard) at 22 days (**Supplementary Table S1**). The growth period for the abovementioned microgreens was generally longer than that reported by previous workers who tended to harvest earlier, at the cotyledonary or first true leaf stages (Pinto et al., 2015; Xiao et al., 2016; Di Gioia et al., 2017). Yield ranged from 1.25 to 5.97 kg fw m^{-2} and significant differences were obtained between species (**Table 1**).

Notably lower yield (<3.0 kg fw m^{-2}) was produced by jute, green basil and cress; highest yield was produced by radish (5.97 kg fw m^{-2}) whereas the rest nine microgreens species yielded 3.01-3.93 kg fw m^{-2}. Overall, higher yield was obtained than previously reported for peat-grown microgreens. For instance, the fresh yield of the four *Brassica rapa* L. subspecies examined in the present study averaged 3.25 kg m^{-2} as opposed to 1.58 kg m^{-2} reported for the related "rapini" (*Brassica rapa* L. - Broccoletto group) microgreens by (Di Gioia et al., 2017). This difference probably reflects the different sowing density (158 vs. 100 g m^{-2}) and the different developmental stage at harvest (appearance of two *vs.* one true leaves). Mean dry matter content of the 13 microgreens species was 5.13%, ranging from 3.89% in mustard to 6.05% in radish. This was overall lower than previously reported; e.g. for the *Brassica rapa* L. subspecies Di Gioia et al. (2017) and Xiao et al. (2016) reported 5.8% and 6.3%, respectively, as opposed to 5.1% (**Table 1**). The lower dry matter content presently obtained likely reflects the more advanced vegetative growth attained at the second-true-leaf stage. Foliage coloration was characterized by lightness (L*) values ranging from 25.64 in dark-colored Pak choi to 42.34 in light-colored Swiss chard. Conversely, as denoted by chroma (C*) values, coloration was most intense in Swiss chard (35.51) and least intense in Pak choi (7.06) (**Table 1**).

Table 1. Fresh yield, dry matter content and canopy colorimatric components L* (lighteness) and C* (chroma) for 13 microgreens species/subspecies grown under controlled environment. All data are expressed as mean ± standard deviation, $n = 3$.

Commercial name	Yield (kg fw m^{-2})	Dry matter (%)	L*	Chroma
Coriander	3.30 ± 0.07 bcd	6.00 ± 0.14 a	32.92 ± 1.55 fg	25.71 ± 1.24 cd
Cress	2.75 ± 0.23 d	5.62 ± 0.57 ab	35.95 ± 0.58 de	28.18 ± 0.58 bc
Green basil	1.62 ± 0.12 e	5.26 ± 0.26 bc	39.12 ± 0.81 bc	29.38 ± 0.72 b
Jute	1.25 ± 0.07 e	5.16 ± 0.02 bcd	41.58 ± 0.37 ab	29.11 ± 0.68 b
Kohlrabi	3.45 ± 0.05 bcd	5.42 ± 0.12 b	38.07 ± 2.31 cd	27.29 ± 2.29 bc
Komatsuna	3.65 ± 0.11 bc	4.56 ± 0.20 e	28.49 ± 2.14 h	14.16 ± 2.21 f
Mibuna	3.71 ± 0.10 bc	4.88 ± 0.03 cde	35.17 ± 0.77 ef	28.23 ± 0.23 bc
Mustard	3.01 ± 0.30 cd	3.89 ± 0.13 f	41.58 ± 0.85 ab	30.10 ± 0.66 b
Pak choi	3.36 ± 0.34 bcd	5.63 ± 0.16 ab	25.64 ± 0.71 i	7.06 ± 0.68 g
Purple basil	3.09 ± 0.19 cd	4.74 ± 0.05 de	30.72 ± 1.87 gh	13.41 ± 3.08 f
Radish	5.97 ± 1.27 a	6.05 ± 0.12 a	32.23 ± 2.20 g	17.26 ± 3.07 e
Swiss chard	3.93 ± 0.15 b	4.10 ± 0.06 f	42.34 ± 1.83 a	35.51 ± 1.70 a
Tatsoi	3.16 ± 0.49 cd	5.33 ± 0.08 bc	32.42 ± 1.55 g	24.17 ± 1.67 d
Significance	***	***	***	***

ns, *** Nonsignificant or significant at $p \leq 0.001$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.2. Nitrate content and mineral composition

From a botanical point of view, nitrate-accumulating vegetable species belong to the families of *Brassicaceae*, *Amaranthaceae* (*Chenopodiaceae*), *Lamiaceae* and *Apiaceae* (*Umbelliferae*) (Colla et al., 2018). This was the case in the current study since significant differences in the mean content of nitrate were observed among the 13 cultivated microgreen species (**Table 2**). Nitrate levels in the harvested microgreens varied considerably across species, with the highest mean nitrate concentration (4488, 5164 and 5386 mg kg^{-1}fw) found in mustard, jute (*Malvaceae*) and kohlrabi, respectively (**Table 2**). Low accumulation of nitrates (<1000 mg kg^{-1}fw) was observed in Swiss chard; moderate levels of nitrates (1000-2600 mg kg^{-1}fw) were observed in purple basil and radish; high levels of nitrates (2600-4000 mg kg^{-1}fw) were recorded in coriander, cress, green basil, komatsuna, mibuna, pak choi and tatsoi. The large variation in nitrate accumulation reported among microgreen species could be associated to genetic factors (Santamaria, 2006), as well as to the differential nitrate

uptake, translocation and accumulation in the vacuoles of the mesophyll cells (Blom-Zandstra, 1989). Although, the nitrate content recorded among the 13 microgreen species was within the maximum nitrate limit for the commercialization of fresh mature vegetables (spinach, lettuce and rocket) according to Commission regulation (EU) No 1258/2011, various approaches should be adopted in the near future to reduce the nitrate content in hyper-accumulator microgreens (green basil, jute, kohlrabi, komatsuna, mustard and pak choi). Effective preharvest approaches may include: (i) the reduction of [NO_3^-] in the nutrient solution (< 2 mM N-NO_3^-) and (ii) a brief nitrate-starvation period implemented by switching to a nitrate-free solution two-to-five days before harvest (Colla et al., 2018).

It has been demonstrated that major minerals in the human diet are crucial to avoid nutritional disorders and disease symptoms due to their well-known potentials and functionalities in the body homeostasis and metabolism (Gharibzahedi and Jafari, 2017). The contribution of vegetables and fruits to dietary intake of K, Na, Ca, Mg and P is estimated at 35%, 11%, 7%, 24% and 11%, respectively (Levander, 2019). Among the macro-mineral constituents studied K was by far the most abundant in the 13 cultivated microgreen species, ranging from 26.2 to 68.7 g kg^{-1} dw, followed by Ca (1.9-32.7 g kg^{-1} dw), P (4.8-8.5 g kg^{-1} dw), Mg (5.7-9.0 g kg^{-1} dw), S (1.6-10.2 g kg^{-1} dw) and finally Na (0.9-9.8 g kg^{-1} dw) (**Table 2**). These findings are in agreement with those of Xiao et al. (2016), who reported that both K and Ca were the predominant macronutrients present in 30 varieties of microgreens, representing 10 species within 6 genera of *Brassicaceae*.

The concentrations of macronutrients in cultivated microgreens revealed significant differences among species (**Table 2**). For instance, K and Na were found in highest concentration in Swiss chard microgreens, however purple basil exhibited the highest Mg content and green basil the lowest Na/K ratio (**Table 2**). The very low ratio recorded in purple and green basil as well as in jute (0.02-0.03) is important from a nutritional point of view, as diets with low Na/K are associated with lower incidence of high blood pressure and strokes (Choi et al., 2011). Limited variation among species was observed with respect to their P content (**Table 2**), which ranged from 4.85 g kg^{-1}dw in mibuna to 8.53 g kg^{-1}dw in jute. Wide variation was observed in sulphur content which ranged from 1.64 g kg^{-1} dw in Swiss chard to 10.28 in kohlrabi. Variation with respect to Ca content was limited, ranging

from 17.43 g kg^{-1}dw in jute to 32.71 g kg^{-1}dw in kohlrabi; however, exceptionally low content (1.99 g kg^{-1}dw) was found in Swiss chard (**Table 2**).

Experimental data on the mineral profile of microgreens species are so far limited (Xiao et al., 2016), thus also limiting data comparison. The difficulty of comparison with previous studies could be attributed not only to the intrinsic species-specific factors of cultivated microgreens, but also to the extrinsic parameters such as growing conditions, nutrient solution composition, growth substrates and harvest stage, which may have affected significantly the mineral content (Kyriacou et al., 2016a). Harvest stage as a source of variation with respect to mineral profile deserves particular attention since radical compositional changes are known to take place during the seed-sprout-microgreens-mature plant continuum (Pinto et al., 2015). While the definition of microgreens indicates the emergence of the first two true leaves as the canonical harvest stage, earlier harvesting at the appearance of the first true leaf or even at the cotyledonary stage might account for significant variability in reported results

Table 2. Nitrate (NO_3-N), phosphorous (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg) and sodium (Na) concentrations of 13 microgreens species/subspecies grown under controlled environment. All data are expressed as mean ± standard deviation, $n = 3$.

Commercial name	NO_3-N (mg kg^{-1} fw)	P (g kg^{-1} dw)	K (g kg^{-1} dw)	S (g kg^{-1} dw)	Ca (g kg^{-1} dw)	Mg (g kg^{-1} dw)	Na (g kg^{-1} dw)
Coriander	2820 ± 241 f	6.64 ± 0.38 c	41.39 ± 1.78 d	2.10 ± 0.09 hi	19.58 ± 0.38 cd	6.19 ± 0.18 cd	4.80 ± 0.07 d
Cress	3106 ± 667 ef	6.79 ± 0.68 c	39.57 ± 2.80 d	8.03 ± 0.96 cd	19.11 ± 2.17 d	5.72 ± 0.77 cd	5.69 ± 0.73 c
Green basil	4032 ± 506 cd	5.90 ± 0.47 d	50.46 ± 2.57 b	2.19 ± 0.23 hi	23.23 ± 1.64 c	6.04 ± 0.58 cd	1.03 ± 0.13 f
Jute	5164 ± 111 ab	8.53 ± 0.10 a	47.92 ± 0.91 b	3.03 ± 0.23 g	17.43 ± 1.09 d	6.49 ± 0.39 c	1.43 ± 0.06 f
Kohlrabi	5386 ± 74.1 a	6.89 ± 0.71 c	41.20 ± 1.50 d	10.28 ± 1.01 a	32.71 ± 0.92 a	7.42 ± 0.30 b	4.78 ± 0.40 d
Komatsuna	3640 ± 174 de	6.59 ± 0.25 c	36.09 ± 1.46 e	7.72 ± 0.22 cd	27.32 ± 0.37 b	5.95 ± 0.09 cd	7.22 ± 0.39 b
Mibuna	2756 ± 460 f	4.85 ± 0.13 e	30.23 ± 2.63 f	6.53 ± 0.68 ef	28.46 ± 5.77 b	5.66 ± 0.29 d	6.91 ± 2.58 b
Mustard	4488 ± 509 bc	5.93 ± 0.48 d	44.42 ± 1.04 c	5.82 ± 0.25 f	29.85 ± 2.06 ab	6.02 ± 0.34 cd	7.01 ± 0.19 b
Pak choi	3855 ± 37.0 cde	7.01 ± 0.21 c	40.05 ± 0.31 d	9.45 ± 0.36 b	23.24 ± 0.25 c	6.43 ± 0.22 cd	5.86 ± 1.37 c
Purple basil	2549 ± 12.3 f	5.07 ± 0.10 e	36.52 ± 1.42 e	2.55 ± 0.13 gh	20.08 ± 2.17 cd	9.04 ± 0.24 a	0.97 ± 0.20 f
Radish	2569 ± 72.3 f	7.82 ± 0.23 b	26.21 ± 0.37 g	8.18 ± 0.18 c	20.03 ± 1.18 cd	5.82 ± 0.18 cd	2.75 ± 0.09 e
Swiss chard	993 ± 24.5 g	8.26 ± 0.05 ab	68.70 ± 0.46 a	1.64 ± 0.02 i	1.99 ± 0.78 e	7.67 ± 0.61 b	9.85 ± 0.56 a
Tatsoi	3294 ± 145 def	5.21 ± 0.03 e	35.65 ± 0.67 e	7.30 ± 0.17 de	19.41 ± 0.87 cd	6.14 ± 0.58 cd	4.70 ± 0.82 d
Significance	***	***	***	***	***	***	***

ns, *** Nonsignificant or significant at $p \leq 0.001$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.3. Antioxidant Activity, Ascorbate, Chlorophyll, and Carotenoid Content

Both the lipophilic and hydrophilic fractions of microgreens exhibited wide variation in their antioxidant activity across the thirteen species examined (**Table 3**). Lipophilic antioxidant activity (LAA) ranged from 303.3 mmol Trolox kg^{-1} dw in jute to 878.3 mmol Trolox kg^{-1} dw in cress. Overall, the LAA was highest in species of the *Brassicaceae*, with the notable exception of purple basil from the *Lamiaceae*. The hydrophilic antioxidant activity (HAA) ranged from 123.5 mmol ascorbate eq. kg^{-1} dw found in jute to 287.4 mmol ascorbate eq. kg^{-1} dw found in kohlrabi, with higher levels in general obtained in species of the *Brassicaceae*. Microgreens content in ascorbic acid also varied widely across species, ranging from 130.9 mg kg^{-1} fw in coriander to 732.7 and 980.4 mg kg^{-1} fw found respectively in cress and purple basil. Similar levels of total ascorbate content were found in commercially available microgreens by Xiao et al. (2012, 2015) in the range of and 106.3-1470.0 mg kg^{-1} fw, with the highest values obtained for radish (*Raphanus sativus* L.) and red cabbage (*Brassica oleracea* L. var. *capitata*). Values reported for mungbean and soybean sprouts were 188.6 and 113.3 mg kg^{-1} fw, respectively (Ebert et al., 2017), which fall in the low range of the above reported levels for microgreens. The higher ascorbate content of microgreens likely relates to the presence of photosynthetic activity, absent from sprouts, since ascorbate is synthesized from photosynthetic hexose products and holds a major role in photosynthesis and photoprotection by regulating the redox state of photosynthetic electron carriers (Smirnoff and Wheeler, 2000).

Significant genotypic variation was demonstrated in terms of microgreens pigmentation expressed in chlorophyll and carotenoid concentrations (**Table 3**). Chlorophyll *a* ranged from 510.0 mg kg^{-1} fw in mustard to 1410.6 mg kg^{-1} fw in pak choi and chlorophyll *b* ranged from 137.3 mg kg^{-1} fw in mustard to 469.4 mg kg^{-1} fw in tatsoi. The mean ratio of chl *a*/chl *b* across species was 3.41, with the lowest ratio (2.85) obtained in mibuna and the highest (4.20) in radish. Total chlorophyll content ranged from 647.3 mg kg^{-1} fw in mustard to 1852.5 mg kg^{-1} fw in pak choi. Previous studies have reported similar levels of chlorophyll *a* and *b* content in microgreens; for instance, Chl *a* and Chl *b* contents in mustard microgreens were reported as 490 and 190 mg kg^{-1} fw, whereas higher levels were found in parsley and lower in beet microgreens (Samuolienė et al., 2017). Similarly, Samuolienė et

al. (2017) determined Chl *a* - Chl *b* concentrations in kohlrabi, mibuna and mustard microgreens as 5.47-2.30, 4.46-2.14 and 3.71-1.51 mg g^{-1} dw, respectively.

In terms of carotenoid concentrations across microgreens species, mean lutein concentration was 396.1 mg kg^{-1} dw while *β*-carotene was 3026 mg kg^{-1} dw (**Table 3**). Lutein levels ranged from 193.5 mg kg^{-1} dw in mustard to 827.9 mg kg^{-1} dw in jute. Much higher variability was observed in the levels of *β*-carotene which ranged from a minimum of 426.1 mg kg^{-1} dw in kohlrabi to as high as 8592.2 mg kg^{-1} dw in green basil. Xiao et al. (2012) reported a lutein concentration range of 13-101 mg kg^{-1} fw, with the lowest found in popcorn shoots and the highest in cilantro, and a *β*-carotene concentration of 6-125 mg kg^{-1} fw, with the lowest found in golden pea tendrils and the highest in sorrel.

Aside from genotypic differences between microgreens species and varieties, variation among reports on carotenoid contents of microgreens relate to differences in spectral quality and light intensity during the growth period and also to the developmental stage of microgreens at harvest (Kopsell et al., 2012; Samuolienė et al., 2017). The potential for enhancing human health-promoting carotenoid concentrations in microgreens by modulating preharvest applications of eustress warrants further research as it may expand the possibilities for enhancing the phytochemical content of this emerging functional food (Rouphael et al., 2018).

Table 3. (LAA) and hydrophillic antioxidant activity (HAA), ascorbic acid, chlorophyll, lutein and *β*-carotene concentrations of 13 microgreens species/subspecies grown under controlled environment. All data are expressed as mean ± standard deviation, $n = 3$.

Commercial name	LAA (mmol Trolox kg^{-1} dw)	HAA (mmol ascorbic ac. eq. kg^{-1} dw)	Ascorbic acid (mg kg^{-1} fw)	Chlorophyll a (mg kg^{-1} fw)	Chlorophyll b (mg kg^{-1} fw)	Total chlorophyll (mg kg^{-1} fw)	Lutein (mg kg^{-1} dw)	*β*-carotene (mg kg^{-1} dw)
Coriander	616.2 ± 80.1 bc	216.0 ± 26.7 bcd	130.9 ± 19 d	699.4 ± 1.45 cd	195.3 ± 1.71 de	894.7 ± 2.96 ef	391.9 ± 42 bcde	8255.0 ± 663 a
Cress	878.3 ± 46.5 a	209.9 ± 12.8 cd	732.7 ± 85 b	697.1 ± 3.71 cd	181.5 ± 3.73 e	878.5 ± 7.22 ef	253.4 ± 37 de	4722.7 ± 370 b
Green basil	688.5 ± 90.4 b	202.6 ± 19.6 de	155.9 ± 16 b	1057.3 ± 8.71 b	285.9 ± 1.60 c	1343.2 ± 10.3 bc	455.1 ± 45 bcd	8592.2 ± 1949 a
Jute	303.3 ± 16.1 e	123.5 ± 1.3 g	165.2 ± 87 d	1076.5 ± 5.77 b	299.7 ± 4.18 bc	1376.2 ± 3.67 bc	827.9 ± 305 a	1542.4 ± 550 cd
Kohlrabi	834.0 ± 10.7 a	287.4 ± 21.9 a	541.2 ± 167 bc	616.6 ± 1.65 d	202.5 ± 3.81 de	819.1 ± 2.16 ef	329.2 ± 39 de	426.1 ± 62 d
Komatsuna	854.1 ± 63.6 a	178.4 ± 15.5 ef	616.8 ± 142 d	1099.5 ± 13.0 b	354.4 ± 2.38 b	1453.9 ± 14.9 b	336.7 ± 135 de	4417.8 ± 1329 b
Mibuna	821.7 ± 67.1 a	238.1 ± 8.6 b	347.8 ± 82 cd	725.0 ± 9.09 cd	254.5 ± 6.24 cd	979.4 ± 14.5 de	308.5 ± 111 de	4645.7 ± 1821 b
Mustard	837.1 ± 48.4 a	177.9 ± 21.3 ef	278.2 ± 43 d	510.0 ± 8.11 d	137.3 ± 0.94 e	647.3 ± 9.03 f	193.5 ± 63 e	2547.8 ± 652 c
Pak choi	864.6 ± 49.4 a	235.0 ± 7.6 bc	527.4 ± 46 bc	1410.6 ± 15.4 a	441.9 ± 6.01 a	1852.5 ± 21.4 a	585.1 ± 119 b	854.7 ± 157 d
Purple basil	783.4 ± 37.4 a	134.7 ± 3.0 g	980.4 ± 310 a	904.5 ± 21.2 bc	283.3 ± 4.05 c	1187.8 ± 25.2 cd	369.3 ± 59 cde	803.7 ± 69 d
Radish	839.9 ± 28.5 a	230.5 ± 11.2 bc	556.8 ± 130 bc	577.6 ± 18.6 d	137.5 ± 0.60 e	715.1 ± 19.2 f	221.4 ± 58 e	438.5 ± 226 d
Swiss chard	429.8 ± 41.5 d	127.8 ± 1.4 g	240.6 ± 129 d	965.1 ± 5.51 b	285.7 ± 2.30 c	1250.8 ± 7.81 bc	316.4 ± 86 de	1287.0 ± 121 cd
Tatsoi	575.5 ± 49.8 c	170.2 ± 8.5 f	560.3 ± 80 bc	1372.4 ± 1.44 a	469.4 ± 0.65 a	1841.8 ± 1.13 a	560.7 ± 76 bc	804.4 ± 37 d
Significance	***	***	***	***	***	***	***	***

ns, *** Nonsignificant or significant at $p \leq 0.001$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.4. Phenolic profiles

The Q Exactive Orbitrap LC-MS/MS analysis of the methanolic extracts of the 13 microgreens species provided a qualitative and quantitative evaluation of their phenolic composition. Total phenolic content differed significantly across microgreens species as it ranged from 691 mg kg^{-1} dw in Swiss chard to 5920 mg kg^{-1} dw in coriander (**Table 4**). Species other than coriander that yielded notably high total polyphenol content were green basil (3506 mg kg^{-1} dw), tatsoi (2645 mg kg^{-1} dw) and mibuna (2586 mg kg^{-1} dw). Xiao et al. (2015) and Bulgari et al. (2017) determined photometrically the total concentration of phenolics in microgreens representing the *Amaranthaceae*, *Brassicaceae*, *Chenopodiaceae* and *Lamiaceae* families and reported respectively as gallic acid equivalents a range of 1.5-7.0 g kg^{-1} dw and 164-328 mg g^{-1} fw, with the highest concentration encountered in *Lamiaceae* (basil) and *Brassicaceae* (rocket). The current study confirmed the high phenolic content of certain *Lamiaceae* microgreens (green basil), although significant varietal differences were also identified, e.g., with respect to purple basil (1166 mg kg^{-1}). The current work moreover highlighted the potential diversity in candidate microgreens species rich in bioactive phenolics, including members of the *Apiaceae* family like coriander.

Twenty-eight phenolic compounds were variably detected and quantitated in the 13 microgreens species examined (**Supplementary Fig. S1, Supplementary Table S2**). Flavonol glycosides represented 67.6% of the mean total phenolic content across species and accounted for 12 of the 28 phenolic compounds quantitated; flavones and flavone glycosides represented 24.8% of the mean total phenolic content across species and accounted for 7 of the 28 phenolic compounds quantitated; hydroxycinnamic acids and their derivatives represented 7.6% of the mean total phenolic content across species and accounted for 9 of the 28 phenolic compounds quantitated. Profiling polyphenols in five species of Brassica microgreens using UHPLC-PDA-ESI/$HRMS^n$, Sun et al. (2013) found a proportional representation of polyphenol groups analogous to the one currently presented for the brassicaceous microgreens (**Table 4**), reporting 105 flavonol glycosides and 30 hydroxycinnamic and hydroxybenzoic acid derivatives out of the 164 polyphenols putatively identified. Previous studies have demonstrated the predominance of *O*-glycosides of kaempferol, quercetin and isorhamnetin as the main flavonols present in mature brassicaceous vegetables (Harbaum et al., 2007; Olsen et al., 2009; Cartea et al., 2011; Li et

al., 2018). The current study demonstrates that these flavonol glycosides are also abundant in the microgreens of brassicaceous vegetables, with the glycosides of kaempherol showing however a more species-related distribution compared to the more ubiquitous interspecific presence of quercetin and isorhamnetin glycosides (**Table 4**).

Polyphenol compositional profiles differed significantly across microgreens species and variation was observed with respect to their major phenolic components. In coriander, key flavonoid components were the 3-*O*-rutinosides of quercetin (rutin), kaempferol and luteolin, with respective mean concentrations of 2392.2, 1315.7 and 1469.6 μg g^{-1} dw, whereas the predominant hydroxycinnamic acid was chlorogenic acid (496.1 μg g^{-1} dw; **Table 4**). In the leaves and stems of mature coriander plants, Barros et al. (2012) found as main flavonoids quercetin-3-*O*-rutinoside (3296.2 μg g^{-1} dw), followed by quercetin-3-*O*-glucuronide (1237.1 μg g^{-1} dw) whereas kaempferol-3-O-rutinoside accounted for only 320.9 μg g^{-1} dw. A similar phenolic profile for mature coriander plants was reported by El-Zaeddi et al. (2017). It is apparent that no radical metabolic transformation of flavonoid components takes place across developmental stages from coriander microgreens to mature plants, with flavonol rutinosides being throughout the major constituents. However, assessment of developmental changes in the total phenolic content from microgreens (5920.2 μg g^{-1} dw; **Table 4**) to mature tissues is hampered by differences in reported contents between Barros et al. (2012) and El-Zaeddi et al. (2017), being 6273.5 and 3282.0 μg g^{-1} dw, respectively.

In Swiss chard, the most abundant phenolic component was isorhamnetin-3-gentiobioside (657.5 μg g^{-1} dw) which was also a key component in mature leaves (Ninfali et al., 2007). However, flavones and their glycosides had minimal presence in microgreens unlike their mature counterparts. Cress microgreens abounded in quercetin-3-*O*-glucoside and kaempferol-7-*O*-glucoside (554.7 and 526.3 μg g^{-1} dw, respectively; **Table 4**). Interestingly, the hydroxycinnamic acids and their derivatives accounted for only 1.4% of the total phenolic content of cress microgreens, present mostly as ferulic, chlorogenic and caffeic acid derivatives. However, a study on cress sprouts Oszmiański et al. (2013) revealed that the phenolic profile of 8-day old sprouts was dominated by sinapic acid and its derivatives with minimal presence of flavonoids, while their total phenolic content averaged a phenomenal 55.8 mg g^{-1} dw. It is inferred from the above that the phenolic profile of cress

might be subject to significant metabolic events during the process of sprouting and beyond the cotyledonary stage that warrant additional research.

Both purple and green basil microgreens were noted for their high relative concentration of flavone derivatives. Purple basil was most abundant in luteolin-malonyl-hexose (261.6 μg g^{-1} dw) and quercetin-malonyl-glucoside (198.5 μg g^{-1} dw), whereas green basil was most abundant in the 7-*O*-glucosides of the flavones luteolin (1478.7 μg g^{-1} dw) and apigenin (1210.1 μg g^{-1} dw; **Table 4**). These glucosides were also present in mature tissues of green basil which were however more abundant in 3-O-glucoside and 3-O-rutinoside of quercetin (Grayer et al., 2002), suggesting that the phenolic transformation of basil incurs significant changes with ontogeny.

In jute microgreens, the highest phenolics were dicaffeoylquinic acid (1111.5 μg g^{-1} dw) and quercetin-malonyl-glucoside (570.5 μg g^{-1} dw). Recent profiling of phenolics in mature jute leaves revealed that their composition as well is characterized by a higher content of hydroxicinnamic acids and their derivatives, mainly caffeoylquinic acid (6736.4 μg g^{-1} dw), and a lower content of flavonoids, most abundant of which was quercetin-3-*O*-rhamnoside (2520.6 μg g^{-1} dw; Ben Yakoub et al., 2018). These results indicate that phenolic constitution in this species does not undergo radical changes across developmental stages although the total phenolic content appears much higher in mature tissues.

In the case of kohlrabi (var. purple Vienna) microgreens, key phenolic components were flavonol-*O*-glycosides, identified mainly as kaempferol-3-*O*-(coumaroyl)-sophoroside-7-*O*-glucoside (675.04 μg g^{-1} dw), kaempferol-3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside (537.2 μg g^{-1} dw) and kaempferol-3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside (235.7 μg g^{-1} dw) (**Table 4**). Sun et al. (2013) also found flavonol-*O*-glycosides to be key polyphenols in a purple kohlrabi variety of microgreens but these were identified as quercetin-3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside and quercetin-3-sinapoyltriglucoside. Varietal differences and even differences in harvest maturity might account for this variation in flavonoid composition (Ebert et al., 2017; Li et al., 2018).

Aside from flavonol glycosides, mature mustard leaves were reported as abundant in hydroxycinnamic acids and their derivatives (Cartea et al., 2011), but their presence in mustard microgreens was minimal compared to the glycosides of kaempferol and quercetin

(**Table 4**). The prominence of quercetin and kaempferol glycosides in the phenolic profile of purple mustard microgreens was also reported by Sun et al. (2013).

Kaempferol and isorhamnetin glycosides were found in abundance in pak choi microgreens and their prominent presence had been previously observed in mature-leaf Pak choi (Cartea et al., 2011). Kampferol and isorhamnetin aglycones (36-1026 and 81-351 μg g^{-1} dw, respectively) were likewise more abundant than the aglycone of quercetin (32-61 μg g^{-1} dw) in young leaves of eleven pak choi varieties (Rochfort et al., 2006). However, unlike their mature-leaf counterparts, pak choi microgreens were most abundant in quercetin-3-*O*-(feruloyl)-sophoroside-7-*O*-glucoside (**Table 4**). Microgreens of the other three varieties of *Brassica rapa* L. (mibuna, tatsoi and komatsuna) varied in their phenolic composition. Mibuna yielded a high concentration of quercetin-3-O-(feruloyl)-sophoroside-7-O-glucoside (1136.8 μg g^{-1} dw) like pak choi, but unlike pak choi it also yielded high concentrations of isorhamnetin-3-gentiobioside (1290.8 μg g^{-1} dw), which was the key phenolic component also of komatsuna (856.8 μg g^{-1} dw). Tatsoi had a distinctly high concentration of kaempferol-3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside (1860.5 μg g^{-1} dw) while it also demonstrated a high concentration of quercetin-3-*O*-(feruloyl)-sophoroside-7-*O*-glucoside (654.3 μg g^{-1} dw), which was the key phenolic component of pak choi (528.4 μg g^{-1} dw). Notwithstanding varietal differences in phenolic composition, differences introduced by the developmental stage of vegetables at harvest appear to be potentially far greater in scale. For instance, using UHPLC-MS/MS Li et al. (2018) profiled the phenolic composition of 12 mature cruciferous vegetables, including two varieties of *Brassica rapa*, var. *chinensis* (Pak choi) and var. *parachinensis* (Choysum) and found that the concentration of hydroxycinnamic acids and their derivatives accounted for 46.8 and 49.1% of their respective total phenolic concentrations, whereas the microgreens of the four *Brassica rapa* varieties analyzed in the present study had a minimal presence of this phenolics category, ranging from merely 0.4% in mibuna to 4.4% in pak choi (**Table 4**). Hence, despite overall differences in phenolic concentrations between microgreens and their mature counterparts, qualitative differences in phenolic composition that impact bioavailability and antioxidant potential must also be considered when comparing vegetables harvested at different stages (e.g., sprouts, microgreens, mature greens; Ebert et al., 2017).

It is evident from the above results that quantitative and qualitative variation in the phenolic composition of microgreens can vary significantly across species but also across intraspecific varieties. Moreover, variation can be found with respect to the developmental stage, and even with respect to leaf position in the case of compact head vegetables, such as *Brassica oleracea* L. (Ferreres et al., 2006). Microgreens are an emerging class of fresh functional foods with an immense potential for enhancing human diet, partly due to their potent phenolic content (Kyriacou et al., 2016a). Understanding the sources of variation in their phenolic composition will be a major contribution to this growing industry, particularly since the bioavailability of the various flavonoids depends on the structure and position of the attached sugar moieties, hence the relative abundance of these compounds does not directly translate to analogous abundance of active metabolites in target tissues (Cartea et al., 2011).

Table 4. Phenolic profiles and total phenolic composition of 13 microgreens species/subspecies grown under controlled environment as detected and quantitated through Orbitrap LC-MS/MS. All data are expressed as mean ± standard deviation, $n = 3$.

Polyphenol (μg g^{-1} dw)	Microgreens												
	Coriander	**Cress**	**Green basil**	**Jute**	**Kohlrabi**	**Komatsuna**	**Mibuna**	**Mustard**	**Pak choi**	**Purple basil**	**Radish**	**Swiss chard**	**Tatsoi**
1,2-disinapoyl-2-feruloylgentiobiose	n.d.	n.d.	n.d.	n.d.	13.50 ± 0.46 b	n.d.	n.d.	n.d.	14.70 ± 1.34 a	n.d.	4.65 ± 0.05 d	3.21 ± 0.00 e	5.84 ± 0.36 c
Apigenin-7-*O*-glucoside	26.41 ± 2.76 bcd	12.16 ± 0.32 cd	1210.11 ± 67.1 a	n.d.	n.d.	22.16 ± 2.13 bcd	24.04 ± 1.57 bcd	39.85 ± 0.74 bc	n.d.	50.02 ± 3.43 b	n.d.	n.d.	n.d.
Caffeic acid	n.d.	n.d.	148.25 ± 9.04 a	3.56 ± 0.05 c	4.15 ± 0.24 c	0.48 ± 0.01 c	0.46 ± 0.16 c	n.d.	5.16 ± 0.22 c	139.68 ± 13.4 b	5.00 ± 0.12 c	3.48 ± 0.02 c	4.49 ± 0.06 c
Caffeic acid hexoside isomers	16.02 ± 0.93 a	4.81 ± 0.50 f	0.16 ± 0.01 g	14.44 ± 1.81 b	9.16 ± 0.41 d	1.00 ± 0.01 g	0.53 ± 0.05 g	0.24 ± 0.02 g	n.d.	12.71 ± 0.43 c	n.d.	6.16 ± 0.10 e	n.d.
Cirsiliol	n.d.	n.d.	n.d.	n.d.	24.55 ± 0.52 a	n.d.	n.d.	n.d.	18.99 ± 1.71 b	1.89 ± 0.03 e	15.03 ± 0.58 c	2.60 ± 0.06 e	9.28 ± 0.37 d
Chlorogenic acid	496.13 ± 38.9 a	7.48 ± 0.08 c	12.01 ± 2.87 c	48.82 ± 4.85 b	n.d.	6.88 ± 0.28 c	6.86 ± 0.94 c	9.35 ± 2.38 c	n.d.	2.86 ± 0.10 c	1.15 ± 0.07 c	1.57 ± 0.05 c	n.d.
Coumaric acid	n.d.	n.d.	n.d.	5.09 ± 0.14 f	7.97 ± 0.55 de	n.d.	n.d.	n.d.	14.52 ± 5.64 b	11.45 ± 0.39 c	25.87 ± 1.48 a	5.31 ± 0.11 ef	9.55 ± 0.19 cd
Dicaffeoylquinic acid isomer1	10.81 ± 0.51 b	n.d.	n.d.	1111.48 ± 220 a	1.00 ± 0.00 b	n.d.	n.d.	n.d.	1.13 ± 0.07 b	1.74 ± 0.07 b	1.24 ± 0.05 b	2.50 ± 0.91 b	1.02 ± 0.01 b
Ferulic acid	0.87 ± 0.07 de	3.82 ± 0.69 c	1.24 ± 0.06 d	3.25 ± 0.01 c	3.55 ± 0.01 c	0.73 ± 0.14 de	1.20 ± 0.07 d	0.43 ± 0.00 e	5.36 ± 0.12 b	3.39 ± 0.03 c	10.75 ± 0.84 a	5.05 ± 0.08 b	5.07 ± 0.23 b
Feruloyl quinic acid isomer 1	52.85 ± 0.80 a	0.04 ± 0.00 e	0.40 ± 0.01 e	5.01 ± 0.64 b	3.81 ± 0.33 c	0.39 ± 0.02 e	0.41 ± 0.01 e	0.40 ± 0.02 e	2.60 ± 1.85 d	n.d.	n.d.	n.d.	n.d.
Isorhamnetin-3-gentiobioside	n.d.	n.d.	n.d.	n.d.	12.22 ± 0.84 d	856.83 ± 345 b	1290.75 ± 222 a	330.79 ± 116 c	79.86 ± 16.9 d	0.28 ± 0.07 d	15.74 ± 1.61 d	657.49 ± 59.4 b	42.46 ± 2.47 d
Kaempferol-3-*O*-(caffeoyl)-sophoroside-7-*O*-glucoside	n.d.	n.d.	n.d.	2.31 ± 1.71 c	235.73 ± 69.6 b	n.d.	n.d.	n.d.	220.25 ± 64.8 b	11.85 ± 0.68 c	n.d.	n.d.	1860.46 ± 87.4 a
Kaempferol-3-*O*-(coumaroyl)-sophoroside-7-*O*-glucoside	n.d.	n.d.	n.d.	n.d.	675.04 ± 22.5 a	n.d.	n.d.	n.d.	n.d.	n.d.	587.14 ± 27.6 b	n.d.	n.d.
Kaempferol-3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside	n.d.	n.d.	n.d.	n.d.	537.16 ± 27.3 a	n.d.	n.d.	n.d.	n.d.	25.48 ± 1.11 c	41.51 ± 0.62 b	0.40 ± 0.01 d	n.d.
Kaempferol-7-*O*-glucoside	n.d.	526.27 ± 17.8 a	n.d.	16.77 ± 4.89 c	6.39 ± 1.74 de	n.d.	n.d.	n.d.	3.59 ± 0.45 de	27.76 ± 0.92 b	1.44 ± 0.07 e	n.d.	11.44 ± 0.76 cd
Kaempferolo-3-*O*-rutinoside	1315.67 ± 34.4 a	19.85 ± 0.52 c	37.69 ± 3.37 b	1.83 ± 0.17 d	n.d.	3.96 ± 0.35 cd	13.11 ± 0.79 cd	7.74 ± 0.22 cd	n.d.	n.d.	n.d.	n.d.	n.d.
Luteolin-3-O-rutinoside	1469.63 ± 145 a	14.83 ± 1.16 c	39.36 ± 1.80 c	1.44 ± 0.33 c	n.d.	3.84 ± 0.27 c	8.28 ± 0.88 c	8.19 ± 0.63 c	n.d.	148.26 ± 4.67 b	n.d.	0.25 ± 0.09 c	n.d.
Luteolin-malonyl-hexose	11.66 ± 1.57 d	n.d.	450.33 ± 88.1 a	92.20 ± 20.9 c	0.48 ± 0.04 d	n.d.	n.d.	n.d.	0.30 ± 0.08 d	261.59 ± 23.3 b	n.d.	0.21 ± 0.01 d	0.32 ± 0.09 d
Luteolin-7-*O*-glucoside	43.79 ± 6.18 bcd	n.d.	1478.70 ± 63.8 a	n.d.	0.33 ± 0.06 d	26.20 ± 1.34 cd	62.91 ± 63.6 bc	85.01 ± 4.32 b	0.45 ± 0.22 d	n.d.	2.44 ± 0.19 d	n.d.	n.d.
Quercetin-3-*O*-galactoside	1.29 ± 0.01 d	n.d.	0.28 ± 0.00 d	n.d.	26.48 ± 3.60 a	0.50 ± 0.34 d	0.90 ± 0.03 d	0.68 ± 0.01 d	6.99 ± 1.42 c	n.d.	22.68 ± 0.87 b	n.d.	n.d.
Quercetin-3-*O*-glucoside	1.37 ± 0.03 e	554.69 ± 8.59 a	0.29 ± 0.01 e	n.d.	24.40 ± 3.36 b	0.62 ± 0.07 e	0.96 ± 0.05 e	0.66 ± 0.03 e	6.46 ± 1.39 d	9.07 ± 0.41 d	18.40 ± 1.04 c	n.d.	n.d.
Quercetin-sophoroside	n.d.	n.d.	n.d.	0.93 ± 0.11 e	6.94 ± 1.49 c	n.d.	n.d.	n.d.	30.89 ± 4.17 a	0.30 ± 0.01 e	4.60 ± 0.58 d	n.d.	26.39 ± 1.95 b
Quercetin-3-*O*-(feruloyl)-sophoroside-7-O-glucoside	8.79 ± 0.40 f	4.58 ± 0.08 f	11.38 ± 1.05 f	2.28 ± 1.14 f	129.09 ± 34.3 e	137.43 ± 7.91 e	1136.80 ± 28.7 a	284.02 ± 4.27 d	528.43 ± 124 c	n.d.	n.d.	n.d.	654.32 ± 63.8 b
Quercetin-3-*O*-sophoroside-7-*O*-glucoside	n.d.	n.d.	n.d.	n.d.	135.66 ± 9.48 a	n.d.	n.d.	n.d.	76.56 ± 27.3 b	n.d.	0.71 ± 0.07 c	n.d.	12.17 ± 1.10 c
Quercetin-malonyl-glucoside	72.27 ± 26.7 c	5.01 ± 0.73 c	34.77 ± 1.30 c	570.54 ± 167 a	0.91 ± 0.03 c	9.29 ± 1.10 c	1.16 ± 0.16 c	6.35 ± 0.92 c	4.96 ± 1.35 c	198.50 ± 13.4 b	11.78 ± 2.31 c	1.20 ± 0.06 c	n.d.
Rosmarinic acid	0.39 ± 0.00 e	0.17 ± 0.00 e	61.72 ± 3.26 a	1.07 ± 0.00 de	3.24 ± 0.25 c	0.55 ± 0.06 e	0.31 ± 0.01 e	0.33 ± 0.04 e	1.85 ± 0.14 cde	43.57 ± 0.33 b	2.75 ± 0.10 cd	1.35 ± 0.03 de	1.89 ± 0.09 cde
Rutin	2392.23 ± 23.6 a	18.32 ± 1.54 d	19.46 ± 0.36 d	1.27 ± 0.06 e	n.d.	5.25 ± 0.29 e	37.05 ± 1.28 c	85.56 ± 8.49 b	n.d.	19.02 ± 1.76 d	n.d.	n.d.	n.d.
Vitexin	n.d.	n.d.	n.d.	5.41 ± 1.75 b	n.d.	n.d.	n.d.	n.d.	n.d.	196.62 ± 13.4 a	1.34 ± 0.25 b	n.d.	n.d.
Total polyphenols	5920 ± 171 a	1172 ± 20 e	3506 ± 189 b	1888 ± 423 d	1862 ± 162 d	1076 ± 349 ef	2586 ± 281 c	860 ± 106 efg	1023 ± 229 efg	1166 ± 74 e	774 ± 23 fg	691 ± 60 g	2645 ± 97 c

n.d. not detected. Different letters within each row indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

Principal Component Analysis (PCA) was performed to obtain a summarized view of the affiliations between the 13 microgreens species examined with respect to their phenolic profiles. The first three Principal Components (PCs) were associated with Eigen values > 1, and explained 59.9% of the total variance, with PC1 accounting for 25.5% and PC2 for 19.7%. The loading matrix illustrates the relationships among the phenolic components identified, with vectors separated by an angle <90° or >90° being positively or negatively correlated, respectively (**Fig. 1A**). The loading matrix indicates three distinct groups of phenolics: the first group in the upper right quadrant of the loading matrix and comprises mainly quercetin and kaempferol glucosides and ferulic and coumaric acids. The clustering of quercetin with kaempferol glucosides relates to the close biochemical relation between the two flavonol glucosides, since hydroxylation of position 3′ on the phenolic group of the 3-hydroxyflavone backbone converts quercetin to kaempferol (Csepregi and Hideg, 2018). The second group in the lower left quadrant and comprises mainly isorhamnetin-3-gentiobioside, flavone glucosides, rosmarinic and caffeic acids. The clustering of rosmarinic acid and caffeic acid in this group is not surprising given that caffeic acid is a product of the enzymatic hydrolysis of rosmarinic acid, which is a main phenolic constituent of the *Lamiaceae* family (Kim and Lee, 2004). The third group in the upper left quadrant andcomprises mainly rutinosides, such as kaempferol, luteolin and quercetin (rutin) rutinosides, and chlorogenic acid. The clustering of the 13 microgreens species based on their phenolic profiles is portrayed in the score plot (**Fig. 1B**) and overall reveals a coincidence of phenolic profiles with botanical families. The *Brassicaceae* members are closely clustered at the center of the matrix, along with Jute (*Malvaceae*) and Swiss chard (*Chenopodiaceae*). The *Lamiaceae* members are positioned in the third quadrant with green basil however distinctly distanced, and finally coriander (*Apiaceae*) demonstrates a distanced phenolic profile to all the rest species. It is indicative in the above clustering that the phenolic profiles of microgreens tend to be largely defined by genotype and therefore adhere closely to their taxonomic origins.

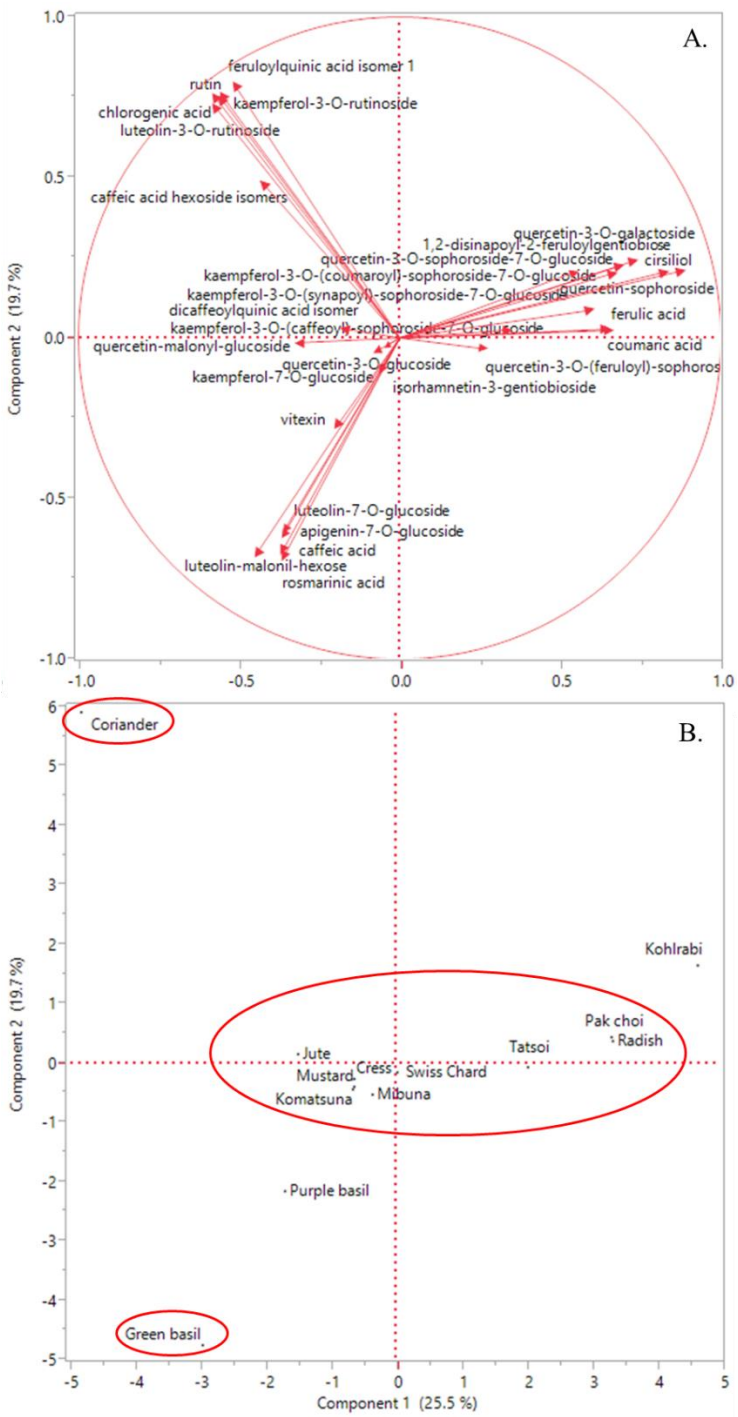

Figure 1. Principal component loading plot (A) and scores (B) of principal component analysis (PCA) of the concentrations of 27 phenolic compounds identified and quantitated by UHPLC-Q-Orbitrap HRMS analysis in 13 microgreens species/subspecies grown under controlled environment.

3.5. Principal Component Analysis on microgreens compositional profiles

A comprehensive view of the compositional profiles of the 13 microgreens species was derived through the PCA of the means of all analytical variables obtained and discussed above (**Fig. 2A and 2B**). The first three PCs were associated with eigenvalues greater than 1 and explained 59.9% of the total variance, with PC1 accounting for 25.5%, PC2 for 19.7% and PC3 for 14.7%. The loading matrix indicates the relations among the examined compositional variables, based on which three groups of positively correlated variables are discerned (**Fig. 2A**): the group in the upper and lower right quadrants comprising nitrate, dry matter content, ascorbate, S, Ca, LAA, HAA and yield; the group in the lower left quadrant comprising P, K, total phenols, carotene and color components L* and C*; the group clustered in the upper left quadrant comprising chlorophyll, lutein and Mg. The score plot (**Fig. 2B**), superimposed on the above matrix of variables (**Fig. 2A**), did not reveal strong clustering of microgreens on a phylogenetic basis as previously observed with the PCA performed solely on phenolic composition (**Fig. 1B**). All brassicaceous microgreens (except tatsoi) and purple basil were positioned on the positive side of PC1 in the upper and lower right quadrants of the PCA score plot, characterized overall by higher nitrate, dry matter, ascorbate, S and Ca contents, antioxidant potential and yield than the rest microgreens (**Fig. 2B**). Jute and tatsoi were positioned in the upper left quadrant, characterized overall by higher chlorophyll and lutein levels relative to the rest species. Finally, in the lower left quadrant were positioned microgreens characterized by high levels of K, color components L* and C* (Swiss chard), and by high contents of β-carotene and total phenolics (coriander and green basil). The above PCA output based on overall compositional profiles does not adhere to taxonomic affiliations among the 13 microgreens species/subspecies, except from a loose clustering of the brassicaceous microgreens driven mostly by their high S, Ca and ascorbate contents. However, an important piece of information emerging from the above two PCA outputs (**Figs. 1 and 2**) is that the phenolic composition commands significant clustering of microgreens compositional profiles. This information is critical for selecting new species/varieties of microgreens since microgreens constitute an emerging gastronomic refinement of high sensory value but also potentially a functional food, rich in bioactive components, phenolic compounds foremost.

It is worth noting that the antioxidant activity accounted for in the loading matrix of **Fig. 2A** correlated positively with ascorbic acid but not with the total phenolics content across species. However, significant correlations with antioxidant activity were obtained for specific phenolic compounds. For instance, HAA correlated significantly with quercetin-3-O-galactoside (r=0.68, $p<0.05$), quercetin-3-O-sophoroside-7-O-glucoside (r=0.64, $p<0.05$), cirsiliol (r=0.64, $p<0.05$) and kaempferol-3-O-(coumaroyl)-sophoroside-7-O-glucoside (r=0.60, $p<0.05$). The fact that total phenolic content did not correlate significantly with antioxidant activity is not surprising and has been reported in the past, particularly in studies involving samples across botanical families, since antioxidant assessment relies on the capacity of selected phenolic compounds in a sample and the type of assay applied (Wojdyło et al., 2007). In the present study the most abundant phenolic compounds found across species were isorhamnetin-3-gentiobioside, quercetin-3-O-(feruloyl)-sophoroside-7-O-glucoside, kaempferol-3-O-(caffeoyl)-sophoroside-7-O-glucoside, rutin and kaempferol-3-O-rutinoside; however, none of this compounds correlated significantly with antioxidant activity.

Phenolic content has been strongly correlated with flavor attributes (sourness, astringency, bitterness) shown to influence consumer acceptability (Xiao et al., 2015). Phenolic content may be prominent in microgreens of less agreeable taste (e.g., red cabbage, sorrel, peppercress), but can be substantial also in species of more agreeable taste (e.g., cilantro and amaranth;Xiao et al., 2012; Kyriacou et al., 2016). The identification of novel microgreen genotypes that satisfy demand for both taste and health remains a challenge since the acceptability of acrid taste is highly varied and subject to both inherited and acquired taste factors (Kyriacou et al., 2016a).

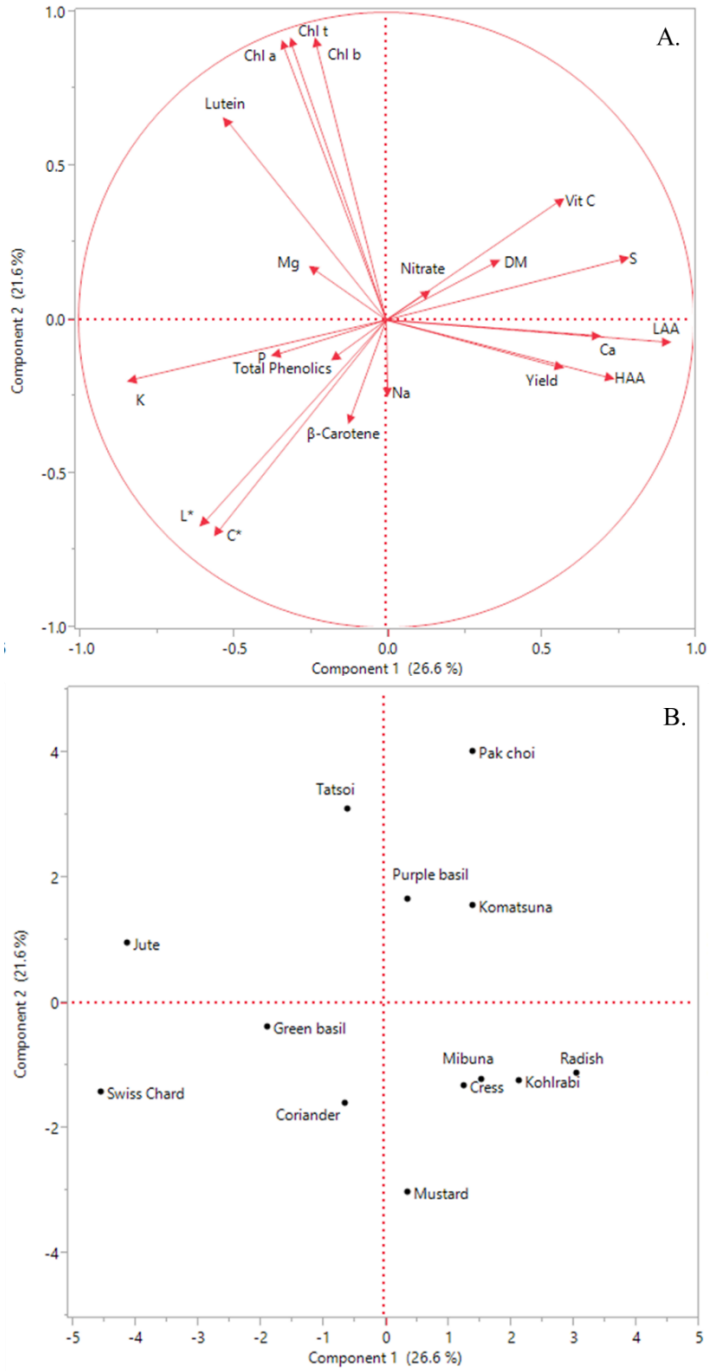

Figure 2. Principal component loading plot (A) and scores (B) of principal component analysis (PCA) of colorimetric data (L*, C*), fresh yield and dry matter content, mineral concentrations (NO_3^--N, P, K, S, Ca, Mg, Na), lipophilic (LAA) and hydrophilic (HAA) antioxidant activity, chlorophyll (a, b, total), lutein, *β*-carotene and total phenolics concentrations in 13 microgreens species/subspecies grown under controlled environment.

4 Conclusions

The brief vegetative growth period required before harvesting microgreens makes genotype selection a key component for this expanding new industry, notwithstanding the potential effects of growth conditions and the developmental stage at harvest. Important compositional differences were presently characterized across microgreens from 13 species and five botanical families. Findings highlighted nitrate hyper-accumulator microgreens warranting preharvest measures to reduce nitrate content, and potassium was found the most abundant macro-mineral, followed by Ca, P, Mg, S and Na. Genotypic differences in Na, K and S concentrations were wide while variation in P, Ca and Mg was narrower. Lipophilic and hydrophilic antioxidant capacities were highest in brassicaceous microgreens. Ascorbate levels in microgreens were found higher than corresponding levels in sprouts, likely due to the presence of photosynthetic hexose precursors absent from sprouts. Genotypic variation was also demonstrated in terms of pigmentation expressed in chlorophyll and carotenoid concentrations. The current study confirmed the high phenolic content of *Lamiaceae* microgreens, with significant however varietal differences; moreover, it highlighted alternative phenolics-rich microgreens, such as coriander from the *Apiaceae*. Qualitative and quantitative determination of phenolic profiles demonstrated the predominance of flavonol glycosides, with the O-glycosides of kaempferol showing more species-related distribution. Though phenolic profiles differed significantly across microgreens, PCA analysis revealed that clustering of phenolic profiles followed closely microgreens' taxonomy. In fact, the phenolic composition is a key determinant of the clustering of microgreens compositional profiles. This information is critical for selecting new species/varieties of microgreens that satisfy demand for both taste and health.

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Chapter 3: Genotype-specific Modulatory Effects of Select Spectral Bandwidths on the Nutritive and Phytochemical Composition of Microgreens

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Genotype-Specific Modulatory Effects of Select Spectral Bandwidths on the Nutritive and Phytochemical Composition of Microgreens

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Genotype-specific Modulatory Effects of Select Spectral Bandwidths on the Nutritive and Phytochemical Composition of Microgreens

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Research Topic: Sprouts, Microgreens and Edible Flowers: Modulation of Quality in Functional Specialty Crops

Abstract

Advanced analytical data on microgreens' response to different light spectra constitutes a valuable resource for designing future crop-specific spectral management systems. The current study defined variation in productivity, nutritive and functional quality (mineral-carotenoid-polyphenolic profiles and antioxidant capacity) of novel microgreens (amaranth, cress, mizuna, purslane) in response to select spectral bandwidths (red, blue, blue-red) and appraised clustering patterns configured by the genotype-light-spectrum nexus. Growth parameters dependent on primary metabolism were most favoured by blue-red light's efficiency in activating the photosynthetic apparatus. Nitrate accumulation was higher under monochromatic light owing to the dependency of nitrite reductase on the light-driven activity of PSI, most efficiently promoted by blue-red light. Although mineral composition was mostly genotype-dependent, monochromatic red and blue lights tended to increase K and Na and decrease Ca and Mg concentrations. Lutein, β-carotene and lipophilic antioxidant capacity were generally increased by blue-red light putatively due to the coupling of heightened photosynthetic activity to increased demand for protection against oxidative stress; the disparate response however of purslane highlights the importance of genotype specificity in these responses and calls for additional investigation. Analysis of polyphenols

by Orbitrap LC-MS/MS revealed substantial genotypic differences. Most abundant phenolics were chlorogenic acid ($\bar{x}$= 5503 μg g^{-1} dw), feruloylquinic acid ($\bar{x}$= 974.1 μg g^{-1} dw) and caffeoylferuloyl tartaric acid ($\bar{x}$= 5503 μg g^{-1} dw). Hydroxycinnamic acids accounted for 79.0% of the mean total phenolic content across species, flavonol glycosides for 20.7% and flavone glycosides for 0.3%. The general response across species was a decrease in individual polyphenolic constituents, particularly flavonol glycosides, and total polyphenols under blue-red light. The pronounced effectiveness of monochromatic blue light in eliciting synthesis of flavonoids could be linked to their capacity for absorbing shorter wavelengths thereby quenching generated photo-oxidation potential. The light-induced stimulation of the phenylpropanoid pathway by monochromatic blue light through epigenetic mechanisms or redox signalling in the photosynthetic apparatus warrants further investigation. The current work highlights how optimized genetic background combined with effective light management might facilitate the production of superior functional quality microgreens.

Keywords: *Amaranth, blue-red light, carotenoids, cress, flavonoids, minerals, mizuna, Orbitrap LC-MS/MS, phenolic compounds, purslane.*

1. Introduction

The promotion of healthy eating remains a topic of prime interest in modern societies (Kyriacou and Rouphael, 2018). As such, consumers are searching for potential nutrient-dense foods that may assist health and longevity (Kyriacou et al., 2016b). Microgreens defined as tender immature greens are a specialty crop gaining popularity due to their fortified plant secondary metabolites (PSM) content, accumulated in their pair of first true leaves, compared to their mature-leaf counterparts (Xiao et al., 2012, 2015; Pinto et al., 2015; Craver et al., 2017). In plant-rich diets, the PSM content imparts beneficial effects to human health, as PSM known to play a primary role in delaying and/or inhibiting oxidative damage, thus preventing a range of common diseases like macular degeneration, cardiovascular diseases and cancer (Kennedy and Wightman, 2011; Khanam et al., 2012; Alrifai et al., 2019).

Although the content and composition of PSM in microgreens varies based on the genetic material (i.e., species), lots of factors are also implicated in modulating PSM,

including cultural practices, conditions of cultivation and environmental factors (Kyriacou et al., 2016, 2017, 2019a). As primary source of energy, light is one of the most important environmental factors along with air temperature for plant growth, development and nutritional quality (Samuolienė et al., 2012; Wang et al., 2016). The appropriate lighting parameters (i.e, intensity, photoperiod and spectral quality) can be optimized and modulated in high-tech greenhouses, plant factories and controlled environment growth chambers using artificial lighting (Vaštakaite et al., 2017; Rouphael et al., 2018). Compared with light intensity and duration, spectral quality shows much more complex responses in terms of crop productivity and functional quality, with mixed findings reported on microgreens (Alrifai et al., 2019).

Considering the importance of red and blue parts of the light spectra in several metabolic pathways and biological processes affecting the metabolism of bioactive compounds (phenolics, carotenoids, ascorbic acids and tocopherols), the Light-Emitting Diodes (LEDs) were introduced in plant cultivation at the beginning of this century as a more efficient light source compared to the most common high-pressure sodium lamps characterized by a high amount of orange-yellow light, with some red and low amount of blue or green spectral components (Son and Oh, 2015; Samuolienė et al., 2016; Samuolienė et al., 2017; Alrifai et al., 2019).

Plants have specialized receptors such as photosynthetic (carotenoid and chlorophyll) and photomorphogenetic (phytochromes and cryptochromes) light receptors, which are responsible for the photophysiological responses induced by light intensity and spectral quality changes, and also modulate several light-sensitive metabolomic/molecular pathways (Samuolienė et al., 2012, 2013; Alrifai et al., 2019). Previous researchers have demonstrated that red light is sensed by phytochromes (PhyA to PhyE) and is responsible for the synthesis of phenolics and antioxidant activity. On the other hand, blue light is sensed by cryptochromes (CRY1 to CRY3) and is implicated in the biosynthesis of anthocyanins, ascorbic acid, chrlorophyll and carotenoids (Li and Kubota, 2009; Olle and Viršile, 2013; Ntagkas et al., 2018).

Over the past few years, spectral effects of red/blue/red-blue bandwidths on PSM have been investigated in microgreens species belonging to the families of *Brassicacceae* (mustard, kale, red pack choi, tatsoi, Kohlrabi, mizuna), *Lamiaceae* (basil and perilla),

Apiaceae (parsely), *Boraginaceae* (borage) and *Chenopodiaceae* (beet, spinach) (Samuolienė et al., 2012, 2016a; Brazaitytė et al., 2015; Craver et al., 2017; Lobiuc et al., 2017). However, information on PSM profiles and how these bioactive compounds respond to LED spectral quality in new and emerging microgreens, like amaranth, cress, and purslane is missing. Since, there is ample evidence of species-specific response to the red/blue/red-blue spectral composition, there is an urgent need among scientists to understand the modulatory mechanism of red and blue light sensing on phytochemical profiles of emerging microgreens, that will definitely lead to the development of species-specific LEDs systems to boost yield and to improve important lipophilic and hydrophilic antioxidant compounds that could be beneficial to the human diet.

In perspective of the above considerations, the objectives of the current study were: i) to evaluate the nutritional and functional composition of new and emerging microgreens (amaranth, cress, mizuna and purslane), ii) to understand the variation in productivity, mineral composition, antioxidant activity, target carotenoids as well as qualitative/quantitative profiles of polyphenols in relation to the light spectra (red and blue LED light percentage) and iii) to appraise possible clustering patterns underlined by species and light quality interaction.

The results displayed in this paper will contribute to the understanding of spectral modulatory effects behind the variation in nutritional and functional quality of select microgreens in demand and will assist the microgreens industry in identifying the optimum species-specific spectral quality systems for achieving fortified plant secondary metabolite content in select microgreens (Ntagkas et al., 2018). The current work may also contribute significantly to the pool of systematic information required to understand how the microgreens chemosphere is configured by the genotype-light-spectrum nexus and consequently how particular species may be streamlined for production under targeted optimal conditions.

2. Materials And Methods

2.1. Chemicals and Standards

Chicoric acid, chlorogenic acid, caffeic acid, catechin, epicatechin, rosmarinic acid, ferulic acid, rutin, vitexin, quercetin-3-*O*-glucoside, lutein and *β*-carotene were obtained

from Sigma (St. Louis, MO, USA). Quercetin-3-*O*-galactoside, kaempferol-7-*O*-glucoside, kaempferol-3-*O*-rutinoside and 3,5-di-*O*-caffeoyl quinic acid were obtaine from Extrasynthese (Genay, France). Methanol and formic acid (LC–MS grade) were obtained from Merck KGaA (Darmstadt, Germany). Ultrapure water was produced by a Milli-Q Gradient A10 water purification system. The purity of the standards was 98%, and all were prepared as initial stock solutions of 1 mg mL^{-1} in methanol. Lutein and *β*-carotene stocks of 1 mg mL^{-1} were prepared in chloroform. Multiple standards stock solutions were prepared as combinations of individual standard stock solutions with further dilutions made with methanol to obtain standard calibration curves in the range of 0.01–5.0 mg L^{-1}.

2.*2. Plant Material and Growth-chamber Conditions*

Four microgreens species were assessed for their bioactive composition: mizuna (*Brassica rapa* var. *japonica* cv. Greens), amaranth (*Amaranthus tricolor* cv. Red garnet), cress (*Lepidium sativum* cv. Curled) and common purslane (*Portulaca oleracea* L.). Seeds of mizuna and amaranth were provided by Condor Seed Production (Yuma, Arizona, USA) while cress and purslane by Nehme Establishment for Trade & Agriculture (Batroun, Lebanon). The sowing density (i), 100-seed weight (ii) and growth cycle duration (iii) for mizuna, amaranth, cress and purslane were respectively: (i) 7, 8, 6 and 8 seeds cm^{-2}, (ii) 172.0, 75.0, 233.6 and 34.7 mg, and (iii) 16, 19, 20 and 21 days after sowing.

Experiments were conducted at the Department of Vegetable Crops of the Agricultural Research Institute (ARI), Nicosia, Cyprus, in controlled-environment Panasonic MIR-554 growth chambers (Panasonic, Gunma, Japan) under Light Emitting Diode (LED) panel units (K5 Series XL750, Kind LED, Santa Rosa, CA, USA). Seeds were germinated in darkness at 24 °C and 100% relative humidity. During the growing cycle day/night temperatures of 22/18 ± 2 °C and RH 65-75% were established. Peat moss was chosen as growth substrate (pH: 6.3, EC: 0.2 dS m^{-1}, porosity: 92% *v/v* and cation exchange capacity: 98 meq 100 g^{-1}) in plastic trays (14×19×6 cm: W× L ×D). One growth chamber with an appropriately tuned LED panel was used to deliver each spectral treatment (red, blue, red-blue). In each chamber, three replicate seeding trays were used for each microgreens species; all trays inside each chamber were rotated daily to ensure absolute uniformity of light interception among replicates.

For fertigation, a quarter-strength Hoagland and Arnon formulation (2.0 mM NO_3-N, 0.25 mM S, 0.20 mM P, 0.62 mM K, 0.75 mM Ca, 0.17 mM Mg, 0.25 mM NH_4-N, 20 μM Fe, 9 μM Mn,0.3 μM Cu, 1.6 μM Zn, 20 μM B, and 0.3 μM Mo) was adopted, accounting for an EC 0.4 ± 0.1 dS m^{-1} and pH 6 ± 0.2. Fertigation was applied manually by means of a laboratory wash bottle instead of foliar spraying (or overhead fertigation) in order to avoid excessive humidity on microgreens stems and leaves. Irrigation volume ranged between 50 and 200 ml/tray/day, alternating between nutrient solution and plain water, in order to maintain the saturation of the substrate and consequently the turgidity of the microgreens. The exact volume depended on the species, the growth stage and the daily evapotranspiration of each tray monitored in terms of the weight loss of each tray between irrigation cycles.

A 12h photoperiod was provided by LED panel units (K5 Series XL750, Kind LED, Santa Rosa, CA, USA) with an emission wavelength range 400-700 nm divided into three customizable channels: red (R) (600-700 nm), blue (B) (400-500 nm) and green-yellow (G) (500-600 nm). The LED panel arrangement inside the growth chamber ensured full coverage of the entire surface of the canopy, providing a homogeneous photosynthetic photon flux density (PPFD) at the canopy level of 300 ± 10μmol m^{-2} s^{-1}. Moreover, the trays were arranged randomly and systematically rotated every 24 h to enhance the uniformity of the light environment. The PPFD and spectral composition were regulated at the beginning and confirmed at the end of each experimental replication run by twelve individual spectral scans per treatment using a spectral radiometer (MSC15, Gigahertz-Optik, Türkenfeld, Germany). Light treatments examined in the present experiment were: red (90% R, 10% G, 0% B), blue (0% R, 10% G, 90% B) and red-blue (45% R, 10% G, 45% B). The PPFD percentage contributions of R, G and B were determined from bandwidth integration; the light spectrum of each treatment is reported in **Figure 1**.

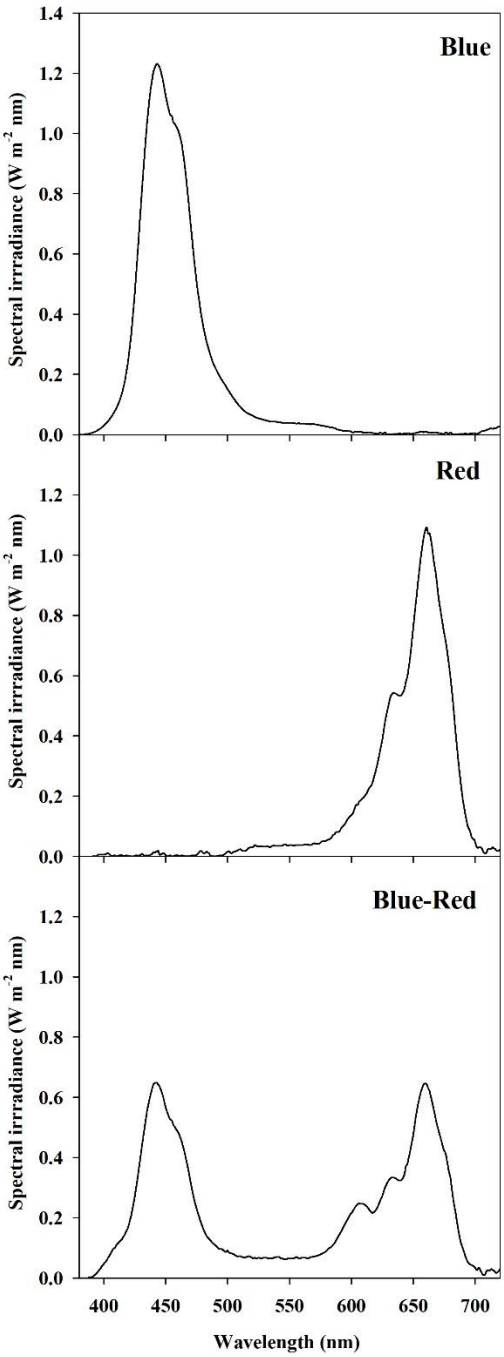

Figure 1. Spectral distribution of light treatments: Red (90% R, 10% G, 0% B), Blue (0% R, 10% G, 90% B) and Red-Blue (45% R, 10% G, 45% B). Spectra were recorded and averaged at twelve locations at the substrate level with a spectral radiometer. Photosynthetic photon flux density for each light treatment was equal to 300 ± 10 μmol m^{-2} s^{-1}.

2.3. Sampling and Fresh Yield Assessment

Just after the appearance of the second true leaf, microgreens were clipped using sterilized scissors just above the substrate level. Fresh yield was determined for each tray and expressed in kg m^{-2}. Determination of nitrate content, mineral composition, lipophilic antioxidant activity, phenolic and carotenoid profiles was performed on fresh samples instantly frozen in liquid nitrogen, stored at -80 ºC before lyophilized in a Christ, Alpha 1-4 (Osterode, Germany) lyophilizer. Determination of ascorbic acid content was performed on fresh subsamples of each tray.

2.4. Dry Matter, Nitrate and Mineral Content Analysis

Dry matter content was determined on triplicate fresh samples following lyophilization to constant weight (≈48 h) measured on a Precisa XT120A analytical balance (Precisa Gravimetrics, Dietikon, Switzerland) and expressed as percentage of microgreens fresh mass. Dry matter samples used for chemical analyses were ground in a Wiley Mill to pass through an 841-microns screen. The concentrations of nitrate, P, K, S, Ca, Mg and Na were determined following sample preparation and ion chromatography as previously described (Kyriacou et al., 2019a). Potassium, Ca, Mg and Na were separated and quantified by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) and electrical conductivity detection. Separation was achieved in isocratic mode on an IonPac CS12A analytical column (4×250 mm, Dionex, Corporation) equipped with an IonPac CG12A precolumn (4×250 mm, Dionex, Corporation) and a self-regenerating suppressor CERS500 (4 mm, Dionex, Corporation). The nitrate, P and S anions were separated using an IonPac ATC-HC anion trap (9×75 mm) and an AS11-HC analytical column (4×250 mm) equipped with an AG11-HC precolumn (4×50 mm) and a self-regenerating suppressor AERS500 (4 mm). Nitrate concentration was converted to mg kg^{-1} fresh weight (fw) based on each sample's original content (dw), while P, K, S, Ca, Mg and Na were expressed as g kg^{-1} dw.

2.5. Assay of Lipophilic Antioxidant Activity

The lipophilic antioxidant activity of microgreens was assessed following extraction procedures previously described (Kyriacou et al., 2019a). Determination of the antioxidant activity in lipophilic extracts employed the 2,20-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS method (Pellegrini et al., 1998). Quantification relied on UV–Vis

spectrophotometry, with the absorbance of lipophilic extracts measured at 734 nm. Trolox was used as the external standard to construct a calibration curve based on six concentration levels (2-20 μmol mL^{-1}) with regression coefficient R^2>0.99. Lipohilic antioxidant activity was expressed in mmol Trolox (6-hydroxy-2,5,7,8-tetramethylchro man-2-carboxylic acid) per kg dw.

2.6. Separation and Quantification of Carotenoids by HPLC-DAD

Carotenoids were extracted from lyophilized samples in ethanol containing 0.1% BHT using a modification of the method of Kim et al. (2008) detailed in Kyriacou et al. (2019). Separation of carotenoid molecules was performed using a Shimadzu HPLC Model LC 10 (Shimadzu, Osaka, Japan) equipped with a reverse phase 250×4.6 mm, 5 μm Gemini C18 column (Phenomenex, Torrance, CA, USA). Injection volume per sample was 20 μL. Acetonitrile (mobile phase A) and ethanol:n-hexane:dichloromethane (1:1:1; mobile phase B) were used to build the following A:B gradient: 0–8 min (82:18); 8-12 min (76:24); 12-18 min (39:61); and 18-25 min a linear gradient to equilibration (82:18). Total runtime was 25 min. Absorbance was measured at 450 nm. Quantification was performed against linear calibration curves built with lutein and *β*-carotene external standards including at least six levels of concentration ranging 5 to 100 μg mL^{-1}.

2.7. Extraction and Analysis of Polyphenols by UHPLC-Q-Orbitrap HRMS

Polyphenols were extracted from lyophilized microgreens (100 mg) using 5 mL methanol/water (60:40, *v/v*) and sonication for 30 min at room temperature. Suspensions were centrifuged at room temperature for 15 min at 4000 rpm and then filtered through a 0.45 μm filter paper (Whatman International Ltd., Maidstone, U.K.). Ten μL of the filtrate were used for mass spectrometry (HRMS-Orbitrap) analysis.

Separation and quantification of polyphenols was performed on an a UHPLC system (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Dionex Ultimate 3000 Quaternary pump performing at 1250 bar and a thermostated (T = 25°C) Kinetex 1.7 μm Biphenyl (100 × 2.1 mm) column (Phenomenex, Torrance, CA, USA). An injection volume of 2 μL was used and eluted at a flow rate of 0.2 mL min^{-1} using a gradient of (A) 0.1% formic acid in H_2O, and (B) 0.1% formic acid in methanol as follows: 0 min - 5% B, 1.3 min - 30% B, 9.3 min - 100% B, 11.3 min - 100% B, 13.3 min - 5% B, 20 min - 5% B.

Mass spectrometry analysis was facilitated by a Q Exactive Orbitrap LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA). All compounds were analyzed using an ESI source (HESI II, Thermo Fischer Scientific, Waltham, MA, USA) in negative ion mode (ESI-) with -2,8 kV spray voltage, sheath gas (N2>95%) 45, auxiliary gas (N2>95%) 10, capillary temperature 275 °C, S-lens RF level 50 and auxiliary gas heater temperature 305 °C. Acquisition of polyphenolic compounds was carried out on parallel reaction monitoring (PRM) mode, with settings as previously described (Kyriacou et al., 2019a). Input time frame for elution and collision energy (CE) were optimized for each polyphenolic compound. A Thermo Fisher Scientific reference standard mixture was used to monitor the accuracy and calibration of the Q Exactive Orbitrap LC-MS/MS. The mass tolerance window was set to 5 ppm for the two analysis modes. Six concentration levels were used to assess linearity at the low (LOQ – 5 mg kg^{-1}) and six at the high (5 mg kg^{-1} – 120 mg kg^{-1}) concentration range. The limits of detection (LODs) and limits of quantitation (LOQs) of the methods were determined. The LOD and LOQ values for the LC-MS/MS analysis of polyphenols were based on chlorogenic acid and rutin signal-to-noise levels. In the case of HPLC-DAD analysis for carotenoids the LOD and LOQ values were determined for *β*-carotene. LOD and LOQ for each compound obtained by serial dilutions of stock solution. Analysis and processing of data were performed using the Xcalibur software, v. 3.0.63 (Xcalibur, Thermo Fisher Scientific).

2.8. Statistics

Two-way analysis of variance (ANOVA) was performed on the experimental data using the SPSS 20 software package. Treatment means were compared using Duncan's Multiple Range Test performed at $P \leq 0.05$. In order to explore relationships among variables and treatments Principal Component Analysis (PCA) was performed (A) on yield, mineral composition, carotenoid composition and total phenolics, and (B) on 13 key phenolic compounds and on total phenolic content of the four microgreens species treated with three light conditions. Principal component analysis was performed using the SPSS 20 software package.

3. Results And Discussion

3.1. Fresh Biomass Yield and Dry Matter Content

The growth period from sowing to full harvest maturity, corresponding to the second true leaf stage, varied considerably for mizuna, amaranth, cress and purslane microgreens, being respectively 16, 19, 20 and 21 days after sowing. Overall, the observed growth periods exceeded those reported for the same species in previous works where harvesting was performed at an earlier (cotyledonary or first true leaf) stage (Pinto et al., 2015; Xiao et al., 2016; Di Gioia et al., 2017).

Fresh biomass yield varied between species (**Figure 2**), with the highest fresh yield obtained from mizuna (2.63-3.52 kg m^{-2}), followed by purslane (2.43-2.87 kg m^{-2}), cress (1.30-2.43 kg m^{-2}) and amaranth (1.24-1.36 kg m^{-2}). In terms of dry mater content however (**Figure 2**), species ranking was inversed, as it was highest in amaranth (6.86-8.31%), followed by cress (5.13-7.37%), purslane (4.68-4.98%) and finally mizuna (4.43-4.47%). The yield obtained across species was overall higher than previously reported (Di Gioia et al., 2017), owing likely to the higher seed density applied in the current work as well as to the longer growth cycle and more advanced growth stage attained before harvest. Regardless of the above overall differences observed between microgreens species with regards to fresh biomass yield and dry matter content, the significant interaction observed between microgreens species (M) and light treatment (L) constitutes an important finding of the present study. This interaction stems from the differential response of the four species to the light conditions applied. In terms of fresh biomass, amaranth demonstrated no response to light treatments (**Figure 2**), as opposed to the rest three species. Both cress and purslane demonstrated the highest fresh yield when grown under blue-red than under red or blue light alone. This type of response was expected since combined blue-red LED bandwidths correspond to the absorption spectra of chlorophyll a and b and have been shown to promote plant growth (Amoozgar et al., 2017). Blue light perception and signaling mediated by cryptochrome photo-receptors is believed to reduce cell wall extensibility and repress cell elongation (Huché-Thélier et al., 2016). However, in the case of mizuna microgreens the highest fresh yield was attained under red light, followed by blue light, while yield was significantly compromised under the blue-red treatment. Ohashi-Kaneko et al. (2007) reported a 39% and 58% reduction in lettuce and spinach dry matter content, respectively,

when grown under blue as opposed to white light. The ability of monochromatic blue and red light to increase biomass is exceptional but has been previously demonstrated on basil (Samuolienė et al., 2016; Alrifai et al., 2019). The underlying mechanism of this species-specific response remains unresolved but is thought to implicate monochromatic light's effect on stomatal conductance and thereby on photosynthetic activity (Huché-Thélier et al., 2016). Two of the species responsive to light treatment (mizuna and purslane) in terms of fresh biomass yield, showed no differentiation as regards their dry matter content. Cress microgreens demonstrated the inverse response to light treatments in terms of fresh yield (blue-red>red=blue) and dry matter content (blue=red>blue-red). Finally, amaranth demonstrated higher dry matter content under the blue-red treatment compared to blue and red treatments alone. The differential growth responses of the four microgreens species possibly reflect blue light's species-specific action on the CRY gene expression, as previously manifested for instance in the downregulation of PsCRY2b expression in *P. sativum* L. and the enhancement of CRY1 expression in *Brassica napus* (Huché-Thélier et al., 2016). Important conclusions derived from the above results are: (a) combined blue-red bandwidths are generally more effective at increasing microgreens fresh biomass production than blue and red bandwidths alone, however species response to variable bandwidths may vary; (b) species responses to bandwidth in terms of fresh biomass yield and dry matter content are generally opposite; for instance, mizuna and purslane seem to readily expend photosynthates in order to fuel rapid cell division taking place at the meristematic regions of their microgreen seedlings, they take up water more efficiently to drive cell expansion and, consequently, they tend to accumulate less dry matter. Differential bandwidth effects on fresh yield and dry matter may reflect different bandwidth efficiencies in activating the photosystems of microgreens (Amoozgar et al., 2017).

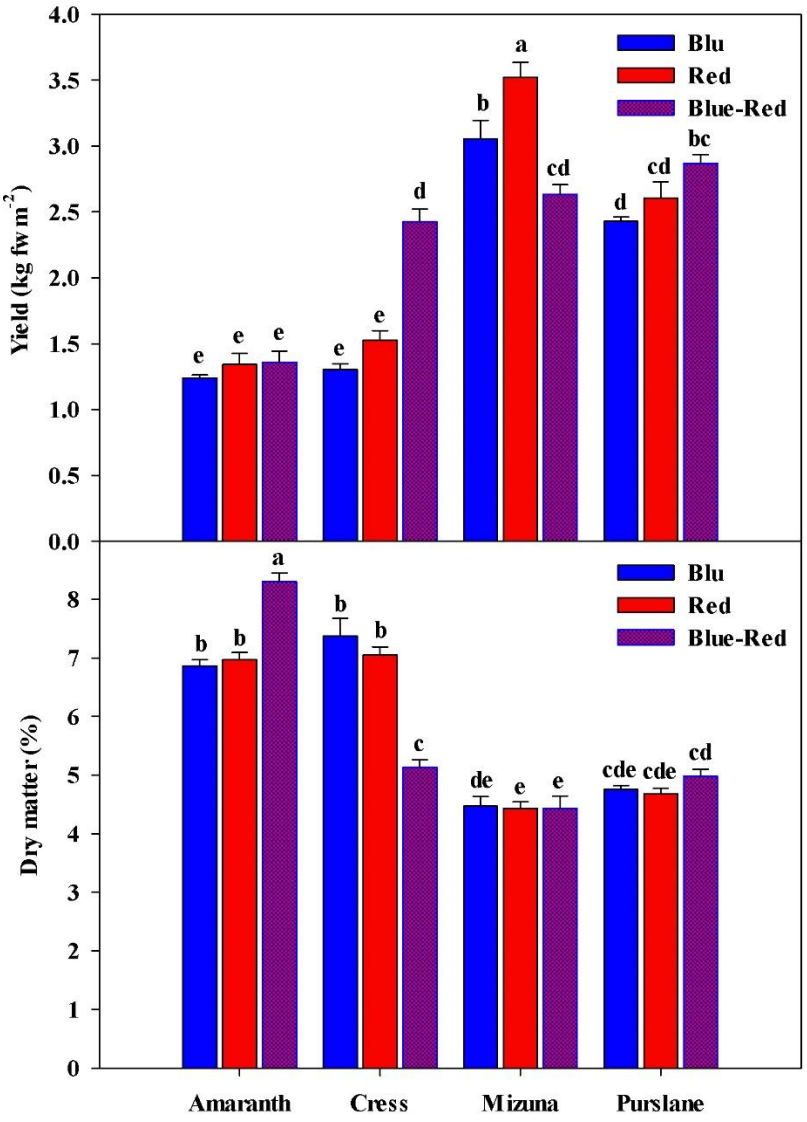

Figure 2. Fresh yield and dry matter of four microgreens genotypes as modulated by variable spectral bandwidths in a controlled growth environment. All data are expressed as mean ± standard error, n = 3.

3.2. Nitrate Concentration and Mineral Composition

Fresh vegetable consumption is the primary source of nitrate intake, high levels of which have been associated with increased probability for carcinogenic nitrosamine formation in the stomach (EFSA, 2008; Colla et al., 2018; Kyriacou et al., 2019a). As a protective measure, the European Commission Regulation Nº 1881/2006 established maximum nitrate

concentrations for three common leafy vegetables: lettuce (3000-5000 mg kg^{-1} fw); spinach (3500 mg kg^{-1} fw); and rocket (6000-7000 mg kg^{-1} fw) (EC, 2006). Little information is so far available for the nitrate levels in microgreens, which constitute an emerging fresh salad component attracting growing culinary and nutraceutical interest. Nitrate concentration among the four microgreens species presently studied varied significantly (**Table 1**). Cress (5074±259 mg kg^{-1} fw) was the highest nitrate accumulator, amaranth (2386±139 mg kg^{-1} fw) accumulated low concentrations of nitrate, whereas mizuna (2856±191 mg kg^{-1} fw) and purslane (3499±238 mg kg^{-1} fw) were moderate accumulators. Nitrate accumulation capacity is a trait strongly associated with plant genetic background and characterizes both mature vegetables and their microgreen counterparts (Kyriacou et al., 2016b). Genetic variation underlines differences in nitrate uptake efficiency, translocation capacity and vacuolar accumulation potential in the mesophyll cells (Blom-Zandastra, 1989). In this respect, the *Brassicaceae*, *Amaranthaceae*, *Lamiaceae* and *Apiaceae* botanical families are the ones generally associated with high nitrate accumulation capacity (Colla et al., 2018; Kyriacou et al., 2019c). However, as indicated by the comparative nitrate levels presently found in cress and mizuna, both members of *Brasicaceae*, nitrate accumulation capacity in microgreens may be subject to considerable genetic variability even across genera of the same family. European Commission regulation (EU) No 1258/2011 defined the maximum allowable levels of nitrate in spinach at 3500 mg kg^{-1} fw, in summer/winter lettuce grown under cover at 5000/4000 mg kg^{-1} fw, and in summer/winter rucola (a known hyper-accumulator from the *Brasicaceae* family) at 7000/6000 mg kg^{-1} fw. Although the four microgreens species examined are not yet regulated with respect to nitrate concentration, moreover microgreens are generally consumed in lower amounts than their mature-leaf counterparts, it is apparent from the present results that the range of concentrations potentially encountered in microgreens can be high, as for instance in cress (4086-5562 mg kg^{-1} fw). Notwithstanding the genetic determinants of nitrate levels in microgreens, several approaches effective at reducing nitrate have been described in previous works (Colla et al., 2018). These include the reduction of nitrate concentration in the nutrient solution and switching to a nitrate-free solution in order to implement nitrate-starvation shortly before harvest.

Aside from the effect of plant genotype, nitrate levels in microgreens were significantly affected by light bandwidth (**Table 1**). The highest nitrate accumulation (3893±347 mg kg^{-}

^{1} fw) was observed under blue light, followed by red (3436±424 mg kg^{-1} fw) and blue-red light (3032±212 mg kg^{-1} fw). However, significant M × L interaction was observed as bandwidth effects were not uniform across species. The blue>red>blue-red effect on nitrate accumulation was more evident on the higher-accumulating species cress and purslane and less so on amaranth and mizuna. However, the general light spectral effect observed was that combined blue-red light was more effective at promoting nitrate assimilation, thus resulting in lower nitrate residual concentrations in microgreens. The capacity of combined blue-red bandwidth to reduce nitrate accumulation likely relates to the higher photosynthetic activity sustained under combined bandwidths that in turn furnishes the carbon and energy necessary for nitrogen assimilation (Champigny, 1995). Combined blue-red bandwidths may maximize the efficiency of Photosystem I that is necessary to drive nitrate reduction (Riens and Heldt, 1992; Ohashi-Kaneko et al., 2007; Qi et al., 2007; Kyriacou et al., 2016b; Colla et al., 2018). Nitrate assimilation in photosynthetic cells is dependent on the tandem reduction of nitrate and nitrite ions. Nitrate reduction takes place in the cytosol through the NADH-specific or NAD(P)H-bispecific action of nitrate reductase and nitrite is subsequently transported to the chloroplast stroma wherein it is further reduced to ammonium by the activity of nitrite reductase (Riens and Heldt, 1992). Nitrite reductase activity is largely light-dependent, driven by the function of PS I, however limited nitrite reduction may rely on reducing agents supplied by catabolic pathways (Colla et al., 2018). Under dark conditions nitrite reductase activity is halted and nitrate reductase activity is also arrested, possibly through feedback inhibition so as to avoid cytotoxic nitrite accumulation (Riens and Heldt, 1992). However, the exact mechanism coordinating the activity of nitrate reductase in the cytosol with photosynthetic activity in the chloroplasts remains largely unresolved (Colla et al., 2018).

The mineral profile of microgreens species has yet received limited attention and existing information from previous studies is unreconciled due to differences in cultivation parameters including growth substrate, nutrient supply and developmental stage at harvest (Xiao et al., 2016). Harvesting prior to the developmental stage defining microgreens, i.e. the second true leaf stage (Kyriacou et al., 2016b), is liable to introduce significant variation to the mineral composition profile of the products, as previously demonstrated during stages of seedlings ontogeny (Pinto et al., 2015). With respect to the concentrations of

macronutrients P, K, Ca, Mg, S and Na significant variation was observed among the four microgreens species examined (**Table 1**). Genotypic differences were most pronounced in terms of Ca (0.59-25.56 g kg^{-1} dw), Mg (5.30-20.17 g kg^{-1} dw), S (3.16-11.99 g kg^{-1} dw) and Na (0.66-12.02 g kg^{-1} dw). More limited genotypic differences were observed with respect to the concentrations of P (6.43-10.08 g kg^{-1} dw) and K (43.23-62.99 g kg^{-1} dw). Purslane exhibited the highest concentrations of P and K, mizuna of Ca and Na, and amaranth of Mg. Both cress and mizuna exhibited distinctly high concentrations of S, which is a signature trait of the glucosinolate-rich *Brassicaceae* microgreens (Neugart et al., 2018). Another important differentiation of genotypes was observed with respect to the Na/K ratio which was notably higher in mizuna (0.28) compared to the rest of the species examined (0.01-0.07). This is a trait of particular interest for human nutrition given the association of low Na/K foods with a lower incidence of high blood pressure and cardiac arrest (Choi et al., 2005).

The incidence of significant M × L interaction indicates differential response to light treatments of the four microgreens species examined with respect to mineral concentrations (**Table 1**). However, the relative contribution of the main effects and their interaction to the variance of mineral concentrations (variance breakdown not shown) indicates that variation is introduced principally by microgreens genotype (M) and much less so by light treatment (L) or the M × L interaction. The effect of light treatment was more pronounced and unambiguous on K and Na concentrations, both of which were significantly higher under the red and blue treatments than under the blue-red light treatment, whereas a generally opposite effect was observed on Ca and Mg concentrations.

Table 1. Nitrate, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and sodium (Na) concentrations of four microgreens genotypes as modulated by variable spectral bandwidths in a controlled growth environment. All data are expressed as mean ± standard error, $n = 3$

Source of variance	Nitrate (mg kg^{-1} FW)	P (g kg^{-1} DW)	K (g kg^{-1} DW)	Ca (g kg^{-1} DW)	Mg (g kg^{-1} DW)	S (g kg^{-1} DW)	Na (g kg^{-1} DW)
Microgreens species (M)							
Amaranth	2386± 139 d	6.48± 0.32 c	47.01± 1.72 c	2.49± 0.13 c	20.17± 0.94 a	4.22± 0.39 c	0.66± 0.08 c
Cress	5074± 259 a	8.27± 0.19 b	52.13± 0.89 b	16.10± 0.47 b	5.30± 0.26 d	11.99± 0.57 a	3.53± 0.28 b
Mizuna	2856± 191 c	6.43± 0.21 c	43.23± 1.02 d	25.56± 0.67 a	7.07± 0.16 c	9.83± 0.23 b	12.02± 1.26 a
Purslane	3499± 238 b	10.08± 0.55 a	62.99± 2.05 a	0.59± 0.04 d	15.28± 1.47 b	3.16± 0.18 d	3.15± 0.34 b
Light quality (L)							
Blue	3893± 347 a	8.33± 0.62 a	54.52± 2.39 a	11.23± 3.10 a	11.02± 1.72 b	7.47± 1.39 a	5.08± 1.61 a
Red	3436± 424 b	7.83± 0.61 a	52.38± 2.80 a	10.45± 2.84 b	10.40± 1.65 b	7.57± 1.20 a	5.29± 1.68 a
Blue-Red	3032± 212 c	7.29± 0.26 b	47.13± 1.95 b	11.88± 3.35 a	14.43± 2.28 a	6.87± 0.81 b	4.16± 0.73 b
M × L							
Amaranth × Blue	2647± 152 def	6.97± 0.27 def	50.50± 2.33	2.75± 0.09 e	19.13± 1.54 bc	2.92± 0.14 ef	0.81± 0.02 e
Amaranth × Red	2125± 248 f	6.16± 0.61 ef	47.28± 2.17	2.68± 0.15 e	18.68± 1.25 c	4.94± 0.22 d	0.75± 0.17 e
Amaranth × Blue-Red	2386± 281 ef	6.31± 0.75 ef	43.26± 3.50	2.05± 0.13 ef	22.69± 1.33 a	4.81± 0.72 d	0.43± 0.03 e
Cress × Blue	5562± 167 a	8.89± 0.20 b	53.63± 0.74	15.37± 0.43 d	4.80± 0.05 f	13.43± 0.49 a	2.75± 0.19 d
Cress × Red	5572± 21 a	8.26± 0.20 bc	53.66± 1.24	15.17± 0.20 d	4.76± 0.07 f	12.63± 0.46 a	3.27± 0.17 d
Cress × Blue-Red	4086± 214 b	7.67± 0.05 cd	49.09± 0.71	17.77± 0.56 c	6.34± 0.14 ef	9.92± 0.08 bc	4.57± 0.18 c
Mizuna × Blue	3215± 401 cd	6.05± 0.08 f	46.79± 0.72	26.09± 0.53 a	6.95± 0.03 ef	10.43± 0.21 b	14.19± 0.51 a
Mizuna × Red	2338± 252 ef	6.03± 0.21 f	42.50± 0.67	23.50± 0.82 b	6.62± 0.15 ef	10.05± 0.18 b	14.74± 0.81 a
Mizuna × Blue-Red	3015± 48 de	7.22± 0.13 cde	40.41± 0.96	27.10± 1.03 a	7.63± 0.15 e	9.01± 0.15 c	7.14± 0.29 b
Purslane × Blue	4149± 103 b	11.40± 0.17 a	67.14± 1.06	0.69± 0.06 fg	13.23± 0.30 d	3.08± 0.23 ef	2.55± 0.04 d
Purslane × Red	3708± 211 bc	10.86± 0.42 a	66.07± 3.01	0.46± 0.05 g	11.53± 0.09 d	2.67± 0.26 f	2.42± 0.16 d
Purslane × Blue-Red	2641± 153 def	7.98± 0.23 bcd	55.76± 0.94	0.61± 0.05 g	21.08± 0.06 ab	3.74± 0.05 e	4.48± 0.10 c
Significance							
Microgreens species (M)	***	***	***	***	***	***	***
Light quality (L)	***	**	***	***	***	*	***
M × L	***	***	ns	***	***	***	***

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.3. Antioxidant Activity and Carotenoid Content

The lipophilic fractions of the four microgreens species examined demonstrated widely variable antioxidant activities (**Table 2**). Antioxidant activity was highest in cress (92.6±2.0 mmol Trolox kg^{-1} dw) and lowest in amaranth (65.7±4.1 mmol Trolox kg^{-1} dw). However, purslane exibited the highest concentrations of lutein (107.0±7.2 mg kg^{-1} dw) and β-carotene (254.3±16.1 mg kg^{-1} dw). Both of these hydrophobic carotenoid molecules have been shown to possess lipophilic antioxidant capacity owing mainly to the conjugated double bonds of their long polyene chain responsible for their light-absorbing properties and the quenching of ROS formed during photosynhesis (Young and Lowe, 2001). As a supplement to human nutrition lutein improves eye protection against short wavelengths, light-induced oxidative damage and macular degeneration (Kvansakul et al., 2006). Beta-carotene demonstrates biological activity as a precursor of vitamin A (Provitamin A) that is essential for growth, immune function and vision.

Aside from genotypic differences in microgreens carotenoids content and lipophilic antioxidant activity, a significant M × L interaction was observed with respect to the above variables. This interaction derived from the differential response of the four species to light treatments. Three of the species examined (amaranth, cress, mizuna) demonstrated significantly higher lutein, β-carotene and lipophilic antioxidant activity when grown under blue-red light than under either bandwidth alone (**Table 2**). Purslane by contrast had the highest lipophilic antioxidant activity and carotenoid content under monochromatic red light than under either blue or combined blue-red light. Such increase in antioxidant capacity in response to monochromatic light has been previously demonstrated with lettuce receiving supplementary or increased blue LED light but the underlying mechanism of this induction and its genotype-specificity remain poorly understood (Johkan et al., 2010). Notwithstanding the genotype-bandwidth interaction, a general conclusion that may be derived from the current work is that combined blue-red light is generally more effective than monochromatic blue or red light in driving an increase in the carotenoid content and the lipophilic antioxidant capacity of most microgreens species. The increase in lutein and β-carotene concentrations and the concomitant increase in antioxidant capacity is likely linked to enhanced photosynthetic activity promoted by the combined bandwidths. Heightened activity of photosystem I could trigger the biosynthesis of additional light-

quenching carotenoid molecules to provide protection from oxidative stress (Saltveit, 2010), as carotenoids have been shown to function as scavengers of Reactive Oxygen Species and as quenchers of singlet oxygen molecules thus protecting photosynthetic apparatus and membrane lipids against oxidative damage (Jahns and Holzwarth, 2012). Previous work has demonstrated that light intensity can influence chloroplast redox status and accumulation of carotenoids, in particular lutein, β-carotene and violaxanthin + neoxanthin, as well as antioxidant enzymes activity (Jahns and Holzwarth, 2012). The sharp increase in both carotenoids and antioxidant capacity observed under blue-red light in three of the four species examined highlights the potential use of spectral management for the production of microgreens fortified with antioxidant phytochemicals and enhanced in functional quality (Ntagkas et al., 2018). Further research is nonetheless warranted to investigate the specificity of such preharvest applications, including genotype-dependent responses, that may constitute valuable tools towards enhancing the nutraceutical value of this emerging functional food (Kyriacou et al., 2019a).

Table 2. Lypophilic antioxidant activity (LAA), lutein and *β*-carotene concentrations of four microgreens genotypes as modulated by variable spectral bandwidths in a controlled growth environment. All data are expressed as mean ± standard error, $n = 3$

Source of variance	LAA (mmol Trolox kg^{-1} DW)	Lutein (mg kg^{-1} DW)	*β*-carotene (mg kg^{-1} DW)
Microgreens species (M)			
Amaranth	65.67 ± 4.1 c	97.2 ± 9.8 b	214.2 ± 24.6 b
Cress	92.58 ± 2.0 a	95.2 ± 7.5 b	218.0 ± 22.3 b
Mizuna	85.91 ± 2.6 b	68.8 ± 5.0 c	164.6 ± 11.8 c
Purslane	84.23 ± 2.6 b	107.0 ± 7.2 a	254.3 ± 16.1 a
Light quality (L)			
Blue	79.36 ± 2.9 b	78.0 ± 3.4 c	198.3 ± 13.1 b
Red	78.92 ± 5.0 b	88.6 ± 9.0 b	186.4 ± 20.4 c
Blue-Red	88.02 ± 2.8 a	109.6 ± 6.4 a	253.6 ± 16.6 a
M × L			
Amaranth × Blue	63.79 ± 1.4 f	79.7 ± 0.5 d	186.4 ± 1.5 de
Amaranth × Red	52.63 ± 1.3 g	76.0 ± 1.4 d	146.7 ± 4.7 h
Amaranth × Blue-Red	80.60 ± 0.5 e	135.9 ± 5.0 a	309.5 ± 4.1 a
Cress × Blue	87.76 ± 1.5 cd	73.9 ± 1.9 d	170.7 ± 2.7 fg
Cress × Red	90.86 ± 2.6 bc	88.7 ± 2.1 c	176.5 ± 5.0 ef
Cress × Blue-Red	99.13 ± 2.2 a	123.1 ± 4.8 b	306.9 ± 2.6 a
Mizuna × Blue	85.42 ± 1.2 d	64.1 ± 0.7 e	164.4 ± 0.6 g
Mizuna × Red	77.69 ± 0.7 e	54.4 ± 1.7 f	123.9 ± 1.9 i
Mizuna × Blue-Red	94.62 ± 2.2 b	87.8 ± 2.3 c	205.4 ± 1.6 c
Purslane × Blue	80.47 ± 0.9 e	94.3 ± 3.0 c	271.7 ± 8.3 b
Purslane × Red	94.49 ± 0.6 b	135.2 ± 3.1 a	298.6 ± 1.9 a
Purslane × Blue-Red	77.72 ± 0.5 e	91.5 ± 1.2 c	192.6 ± 1.7 d
Significance			
Microgreens species (M)	***	***	***
Light quality (L)	***	***	***
M × L	***	***	***

*** significant at $p \leq 0.001$, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.4. Phenolic Profiles

Chromatographic separation and quantitation by Q Exactive Orbitrap LC-MS/MS analysis of the polyphenols extracted from the four microgreens species treated with variable spectral bandwidths, revealed substantial differences of genotypic origin. In terms of total polyphenols, calculated as the sum of the individual polyphenols quantitated, purslane had the highest content (13581±182 μg g^{-1} dw) followed closely by amaranth (12825±330 μg g^{-1} dw), whereas mizuna (5122±212 μg g^{-1} dw) and cress (4354±156 μg g^{-1} dw) had notably lower polyphenolic contents. Similar levels of polyphenols in microgreens were previously reported by Xiao et al. (2015; 1500-7000 μg g^{-1} dw) and by Bulgari et al. (2017; 164-328 μg g^{-1} fw), determined however by spectrophotometry using gallic acid as the external standard. The predominance of chlorogenic acid, which accounts for most of the total phenolic content presently found in purslane and amaranth microgreens, has been previously found in several *Brassica oleracea* L. cultivars at the seedling stage (Vallejo et al., 2003) and has been described as one of the most efficient and abundant antioxidant products of the phenylpropanoid pathway in young plant tissues (Saltveit, 2010).

Twelve principal phenolic compounds extracted from the four microgreens species examined in the present study were identified and quantified (**Table 3**). In terms of relative content (percentage of total phenolic content), the most abundant phenolic compounds across species were chlorogenic acid (998-10125 μg g^{-1} dw), feruloylquinic acid (971-983 μg g^{-1} dw) and caffeoylferuloyl tartaric acid (970-1056 μg g^{-1} dw). Overall, of the 12 principal phenolic compounds quantified, hydroxycinnamic acids and their derivatives accounted for 79.0% of the mean total phenolic content across species, flavonol glycosides for 20.7% and flavone glycosides for 0.3%. However, significant qualitative differences were identified between species with respect to their phenolic profiles. Significantly higher concentrations of signature phenolics were identified in certain species: apigeninmalonyl glucoside, caffeic acid, p-coumaric acid and kaempferol-3-*O*-synapoil-sophoroside-7-*O*-glucoside were highest in mizuna microgreens; feruloylglycoside was highest in cress; whereas chlorogenic acid was distinctively higher in purslane and amaranth compared to cress and mizuna. Previous studies have reported on the relative abundance of flavonols (e.g., kaempferol, quercetin and isorhamnetin glycosides) in mature vegetables of cruciferous species (Cartea et al., 2011; Li et al., 2018). However, the microgreens of

cruciferous species examined in the present study were less abundant in flavonol glycosides, accounting for only 26.8% and 33.1% of polyphenols in cress and mizuna, respectively (**Table 3**). In fact, the phenolic profiles of all four species examined were dominated by hydroxycinnamic acids and their derivatives. This difference might be attributed to the dramatic compositional changes taking place during the developmental stages of vegetable species from seed to mature vegetable. Previous work profiling the phenolic composition of broccoli (*Brassica oleracea* L.) along progressive developmental stages has demonstrated the relative abundance of chlorogenic acid during the young seedling stage and its progressive decrease relative to the total flavonoids content, despite the rise in absolute concentration (Vallejo et al., 2003). Similarly, the phenolic profile of cress sprouts abounded in hydroxycinnamic acids and their derivatives, as compared to flavonoids (Oszmiański et al., 2013). Developmental transformation of phenolic compositional profile is apparently more pronounced for certain species than others; for instance, coriander (*Coriandrum sativum* L.) from microgreens to mature plant does not seem to undergo radical phenolic transformation, dominated throughout by flavonol glycosides (Barros et al., 2012; El-Zaeddi et al., 2017). The same is true for jute that was shown to maintain a similar phenolic fingerprint high in hydroxycinnamic acids and their derivatives from microgreens (Kyriacou et al., 2019a) to mature plant (Ben Yakoub et al., 2018). It can be therefore hypothesized that the profile and concentration of phenolics in plants during ontogeny depends highly on their genetic constitution, with examples of both interspecific and intraspecific (same species cultivars) already demonstrated (Vallejo et al., 2003; Kyriacou et al., 2019a).

Aside from the genotypic factor, significant differences in the microgreens phenolic composition were also observed in response to light treatments (**Table 3**). A significant M × L interaction characterized variation in almost all the phenolic components quantified, including the total phenolic content. These interactions however stemmed mostly from changes in scale rather than changes in species rank with respect to their response to light treatments. The general response pattern observed across species was a decrease in the concentration of individual phenolic compounds and the total phenolic content when microgreens were grown under combined blue-red bandwidths compared to either bandwidth alone. This decline was particularly sharp in the concentrations of flavonol glycosides, amounting to 67.4%, 48.6%, 53.8% and 42.7% decrease in feruloylglycoside,

kaempferol-3-*O*-synapoil-sophoroside-7-*O*-glucoside, kaempferolo-3-*O*-rutinoside and rutin, respectively, compared to the mean respective concentrations observed under blue and red light (**Table 3**). The total phenolic content under blue-red light declined by 13.3% compared to the mean content observed under blue and red light.

Previous works have pointed to the influential role of monochromatic blue and red lights, compared to white light, on the synthesis and accumulation of secondary metabolites (Wu et al., 2007; Li and Kubota, 2009; Alrifai et al., 2019). The concerted activation of photoreceptors by LED lights has been hypothesized to influence the up- or down-regulation of enzyme activities responsible for the biosynthesis of plant secondary metabolites, many of which might constitute key bioactive components (Qian et al., 2016; Alrifai et al., 2019). Different spectral bandwidths were found variably influential on the synthesis, accumulation and biodegradation of bioactive compounds in lettuce and on chloroplast configuration and photosynthetic performance (Muneer et al., 2014). The current findings are intriguing as they highlight the potential biostimulatory role of blue light, which is sensed by cryptochromes (CRY1 to CRY3) and implicated according to previous studies in the biosynthesis of anthocyanins, chrlorophyll and carotenoids (Li and Kubota, 2009; Olle and Viršile, 2013). Although the same researchers supported that red light, sensed by phytochromes (PhyA to PhyE) is chiefly responsible for the synthesis of phenolics, the current work indicates that blue light alone can support the synthesis of phenolic compounds. Targeted increase of flavonol levels by postharvest exposure to blue light has been previously demonstrated on onion bulbs (Ko et al., 2015). Similarly, Taulavuori et al. (2013) reported a 662% and 96% increase in chlorogenic acid content of basil and tomato, respectively, when gown under supplemental blue light vs. HPS light alone. However, significant differences among species as regards their sensitivity to blue light have been reported (Snowden, 2015). The presence of energy-rich shorter wavelengths alone might elicit the increased biosynthesis of flavonoids in order to quench the higher photo-oxidation potential generated. Recent work has demonstrated that blue light may exert oxidative stress in *Arabidopsis* potentially expressed as damage to photosynthetic apparatus, DNA, lipids and proteins in ways previously seen in response to UV radiation (El-Esawi et al., 2017). Corroborating evidence from recent works suggests that blue light elicits the biosynthesis of phenolic acids and flavonoids in plants by triggering the expression of key enzymes in the

phenylpropanoid pathway in order to counteract oxidative stress, although the species-specificity underscoring these transcriptional responses remains under-characterized and poorly understood (Kojima et al., 2010; Huché-Thélier et al., 2016; Hasan et al., 2017; Kitazaki et al., 2018; Taulavuori et al., 2018). Experiments with *Arabidopsis* photoreceptor mutants have confirmed that blue light-mediated CRY photoreception systems control chalcone synthase expression (Jenkins et al., 2001). Blue light's species-specific action on CRY gene expression seems key to unravelling plant species-specific responses to monochromatic light (Huché-Thélier et al., 2016).

It is nonetheless intriguing why the simultaneous exposure of microgreens to short (blue) and long (red) wavelengths compromises the effects of either bandwidth alone. It is possible that exposure to both bandwidths might promote the photoisomerization of phenolic compounds thus complicating the quantification process (Saltveit, 2010). The efficacy of light-induced effects in conferring physiological and compositional changes to plants, including the stimulation of the phenylpropanoid pathway, has been previously demonstrated; however, the nature of these effects is contested between supporters of epigenetic mechanisms and those proposing changes in the PS II photochemistry mediated by redox signaling in the photosynthetic apparatus (Ganguly et al., 2018; Sytar et al., 2019).

Table 3. Phenolic profiles and total phenolic composition of four microgreens genotypes as modulated by variable spectral bandwidths in a controlled growth environment. All data are expressed as mean ± standard error, $n = 3$

Source of variance	Apigenin malonyl glucoside (µg g^{-1} DW)	Caffeic acid (µg g^{-1} DW)	Chlorogenic acid (µg g^{-1} DW)	p-coumaric acid (µg g^{-1} DW)	Ferulic acid (µg g^{-1} DW)	Feruloyl quinic acid (µg g^{-1} DW)	Feruloylglycoside (µg g^{-1} DW)	Caffeoyl feruloyl tartaric acid (µg g^{-1} DW)	Kaempferol-3-O-synapoil-sophoroside-7-O-glucoside (µg g^{-1} DW)	Kaempferol-7-O-glucoside (µg g^{-1} DW)	Kaempferolo-3-O-rutinoside (µg g^{-1} DW)	Rutin (µg g^{-1} DW)	Total polyphenols (µg g^{-1} DW)
Microgreens species (M)													
Amaranth	4.14 ± 0.1 c	12.78 ± 2.5 bc	9778 ± 12 b	13.8 ± 1.9 c	31.37 ± 3.47 c	970.7 ± 0.27 b	109.3 ± 15.01 b	970 ± 0.04 c	nd	52.55 ± 5.07 a	110.2 ± 11.40 b	1105 ± 124	12825 ± 330 b
Cress	32.86 ± 7.5 b	9.85 ± 1.8 c	998 ± 2 d	47.8 ± 16.8 b	29.29 ± 5.00 b	971.1 ± 0.28 b	129.0 ± 36.66 a	972 ± 0.56 b	9.79 ± 2.1 b	32.29 ± 2.61 d	37.7 ± 8.03 c	1085 ± 152	4354 ± 156 d
Mizuna	61.51 ± 6.3 a	93.77 ± 19.3 a	1112 ± 13 c	89.5 ± 8.9 a	45.36 ± 6.15 a	983.3 ± 1.93 a	72.9 ± 12.35 c	971 ± 0.41 b	1515 ± 154 a	45.42 ± 3.03 b	132.7 ± 30.82 a	nd	5122 ± 212 c
Purslane	36.86 ± 3.7 b	17.11 ± 1.9 b	10125 ± 54 a	8.7 ± 0.7 c	30.10 ± 2.87 b	971.2 ± 0.47 b	61.0 ± 5.21 d	1056 ± 17.62 a	nd	41.42 ± 0.69 c	135.7 ± 7.79 a	1098 ± 107	13581 ± 182 a
Light quality (L)													
Blue	38.73 ± 9.1 a	37.10 ± 9.6 b	5527 ± 1355 a	62.2 ± 15.2 a	43.62 ± 4.30 a	974.7 ± 1.52 a	128.3 ± 24.95 a	1007 ± 18.14 a	874.56 ± 385 a	49.52 ± 5.16 a	149.8 ± 20.59 a	1060 ± 92 b	9250 ± 1320 b
Red	41.11 ± 6.9 a	50.25 ± 19.4 a	5546 ± 1348 a	26.0 ± 5.4 c	35.57 ± 3.57 b	975.3 ± 2.10 a	111.7 ± 10.30 b	995 ± 12.49 b	944.52 ± 422 a	42.00 ± 0.44 b	103.8 ± 12.93 b	1496 ± 61 a	9521 ± 1293 a
Blue-Red	21.68 ± 4.7 b	12.78 ± 2.9 c	5436 ± 1323 b	31.7 ± 12.4 b	20.09 ± 0.47 c	972.1 ± 1.65 b	39.1 ± 4.53 c	977 ± 3.54 c	467.92 ± 207 b	37.25 ± 1.96 c	58.6 ± 10.71 c	732 ± 31 c	8140 ± 1237 c
M × L													
Amaranth × Blue	4.35 ± 0.1 e	22.41 ± 1.0 cd	9803 ± 5 c	16.1 ± 0.4 de	23.60 ± 0.00 e	971.1 ± 0.27	100.8 ± 4.31 cd	971 ± 0.03 d	nd	72.81 ± 0.31 a	118.9 ± 8.40 d	1389 ± 39 c	13493 ± 37 c
Amaranth × Red	4.38 ± 0.2 e	10.04 ± 0.2 efg	9798 ± 6 c	19.1 ± 0.5 d	39.14 ± 0.06 c	971.3 ± 0.14	164.6 ± 4.71 b	970 ± 0.06 d	nd	42.84 ± 0.16 c	143.6 ± 1.13 c	1309 ± 21 c	13473 ± 26 c
Amaranth × Blue-Red	3.68 ± 0.0 e	5.88 ± 0.1 g	9734 ± 6 d	6.3 ± 0.1 e	nd	969.7 ± 0.07	62.5 ± 0.03 e	nd	nd	42.00 ± 0.20 cd	68.0 ± 1.79 f	616 ± 24 f	11508 ± 29 e
Cress × Blue	23.02 ± 1.5 d	17.02 ± 1.2 def	1002 ± 1 g	114.3 ± 7.1 a	49.28 ± 0.33 b	972.1 ± 0.35	268.6 ± 15.15 a	974 ± 0.28 d	18.03 ± 0.3 d	27.40 ± 0.52 f	66.4 ± 3.41 f	766 ± 13 e	4297 ± 16 h
Cress × Red	62.37 ± 1.4 b	6.67 ± 0.4 fg	999 ± 3 g	17.8 ± 1.0 de	19.72 ± 0.04 f	970.7 ± 0.04	94.5 ± 6.50 d	972 ± 0.10 d	6.17 ± 0.1 d	42.69 ± 0.48 c	35.3 ± 0.30 h	1689 ± 55 a	4916 ± 61 g
Cress × Blue-Red	13.18 ± 0.6 e	5.85 ± 0.0 g	992 ± 3 g	11.4 ± 0.8 de	18.87 ± 0.55 f	970.4 ± 0.05	24.0 ± 1.07 g	970 ± 0.11 d	5.16 ± 0.1 d	26.78 ± 0.50 f	11.3 ± 0.42 i	800 ± 25 e	3849 ± 23 i
Mizuna × Blue	84.32 ± 7.5 a	91.19 ± 8.3 b	1070 ± 8 f	109.9 ± 7.0 ab	62.28 ± 2.54 a	983.3 ± 0.66	77.5 ± 3.60 e	972 ± 0.13 d	1731 ± 88.5 b	56.92 ± 1.59 b	251.6 ± 5.54 a	nd	5491 ± 93 f
Mizuna × Red	54.58 ± 1.6 b	160.92 ± 7.9 a	1160 ± 5 e	56.2 ± 3.6 c	52.00 ± 2.06 b	987.3 ± 0.52	112.8 ± 4.25 c	972 ± 0.07 d	1883 ± 116 a	42.37 ± 0.98 cd	100.7 ± 3.42 e	nd	5581 ± 109 f
Mizuna × Blue-Red	45.64 ± 1.5 c	29.22 ± 0.9 c	1106 ± 5 f	102.5 ± 5.7 b	21.81 ± 0.23 ef	979.1 ± 5.19	28.4 ± 1.38 fg	969 ± 0.01 d	931 ± 37.7 c	36.97 ± 0.32 e	45.8 ± 1.58 g	nd	4295 ± 30 h
Purslane × Blue	43.23 ± 2.2 c	17.79 ± 0.2 de	10233 ± 24 a	8.6 ± 0.1 de	39.31 ± 0.05 c	972.3 ± 0.38	66.3 ± 1.26 e	1111 ± 5.61 a	nd	40.94 ± 1.56 cd	162.4 ± 0.91 b	1024 ± 41 d	13719 ± 56 b
Purslane × Red	43.11 ± 6.0 c	23.37 ± 0.9 cd	10227 ± 40 a	11.0 ± 0.1 de	31.42 ± 0.02 d	971.9 ± 0.22	74.9 ± 4.95 e	1067 ± 3.56 b	nd	40.07 ± 0.67 d	135.6 ± 3.95 c	1490 ± 75 b	14115 ± 116 a
Purslane × Blue-Red	24.22 ± 2.0 d	10.18 ± 0.1 efg	9914 ± 3 b	6.5 ± 0.1 e	19.59 ± 0.02 f	969.4 ± 0.03	41.6 ± 0.55 f	991 ± 0.96 c	nd	43.25 ± 0.43 c	109.1 ± 0.61 de	780 ± 14 e	12908 ± 15 d
Significance													
Microgreens species (M)	***	***	***	***	***	***	***	***	***	***	***	ns	***
Light quality (L)	***	***	***	***	***	*	***	***	***	***	***	***	***
M × L	***	***	***	***	***	ns	***	***	***	***	***	***	***

ns,*, *** Nonsignificant or significant at $p \leq 0.05$ and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.5. Principal Component Analysis of Polyphenolic Composition of Microgreen Species Grown under Variable Bandwidths

Principal Component Analysis (PCA) was performed on polyphenolic profile and on total phenolic content of microgreens species grown under variable bandwidths (**Figure 3**).

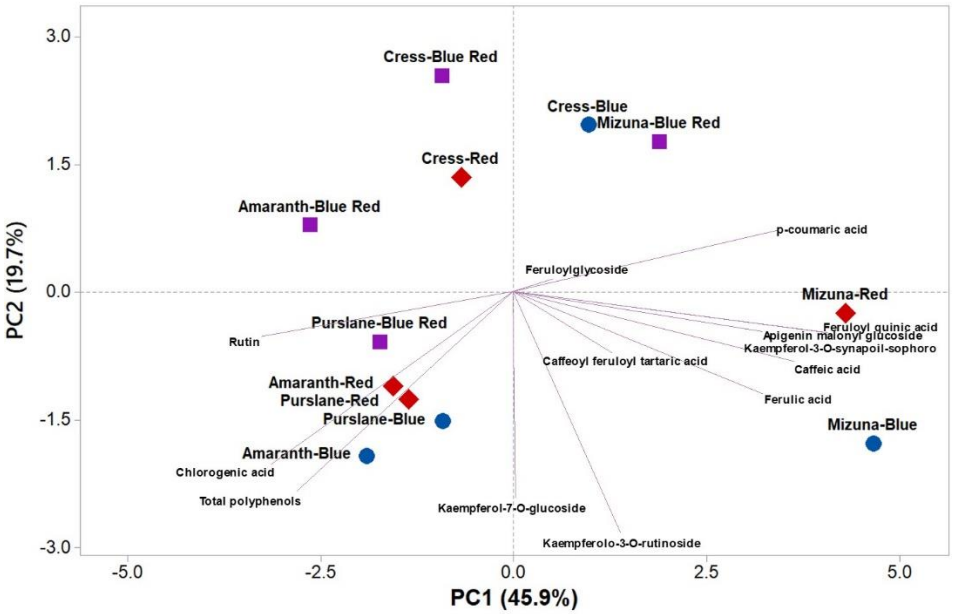

Figure 3. Principal component loading plot and scores of principal component analysis (PCA) of the concentrations of 13 key phenolic compounds and total polyphenols identified and quantitated by UHPLC-Q-Orbitrap HRMS analysis in four microgreens genotypes as modulated by variable spectral bandwidths in a controlled growth environment.

The first three principal components (PCs) were related with eigen values higher than one and explained 79.2% of the total variance, with PC1, PC2, and PC3 accounting for 45.2%, 22.0% and 12.0%, respectively (**Supplementary Table S1)**. The first PC was positively correlated to kaempferol-3-*O*-synapoil-sophoroside-7-*O*-glucoside, feruloyl quinic acid, caffeic acid, p-coumaric acid, ferulic acid and apigenin malonyl glucoside (**Supplementary Table S1**). PC1 was also negatively correlated to rutin, chlorogenic acid and total polyphenols. Moreover, PC2 was positively correlated to kaempferolo-3-*O*-rutinoside, kaempferol-7-*O*-glucoside and total polyphenols (**Supplementary Table S1**).

Supplementray Table 1. Eigen values, relative and cumulative proportion of total variance, and correlation coefficients for polyphenols profile in four microgreens genotypes as modulated by variable spectral bandwidths with respect to the three principal components.

Principal components	**PC1**	**PC2**	**PC3**
Eigen value	5.8	2.8	1.5
Percentage of variance	45.2	22.0	11.9
Cumulative variance	45.2	67.2	79.1
Eigen vectors [a]			
Kaempferol-3-O-synapoil-	**0.943**	0.218	-0.190
Feruloyl quinic acid	**0.932**	0.222	-0.140
Rutin	**-0.849**	0.035	0.171
Caffeic acid	**0.826**	0.317	-0.086
p-coumaric acid	**0.788**	-0.200	0.345
Chlorogenic acid	**-0.724**	0.606	0.044
Ferulic acid	**0.684**	0.436	0.517
Apigenin malonyl	**0.672**	0.222	-0.303
Kaempferolo-3-O-	0.233	**0.893**	0.174
Total polyphenols	**-0.669**	**0.704**	0.054
Feruloylglycoside	0.086	-0.104	**0.964**
Caffeoyl feruloyl tartaric	-0.375	0.525	-0.123
Kaempferol-7-O-glucoside	-0.009	**0.635**	-0.081

[a]Boldface factor loadings are considered highly weighed

Furthermore, the loading matrix indicates the correlations among the examined 13 key phenolic compounds and total phenolic content. In the current study for instance, variation in chlorogenic acid and caffeoyl feruloyl tartaric acid was most closely aligned with total polyphenols content, whereas variation in p-coumaric acid was not correlated to total phenolics (**Figure 3**).

The score plot of the PCA integrated useful information on the polyphenolic profile of the tested microgreens genotypes grown under monochromatic red or blue, or dichromatic red and blue LED lighting. The mizuna microgreen grown under blue and to a lesser extent under red LED lighting was positioned on the positive side of PC1 in the lower right quadrant of the PCA score plot as it delivered microgreens of premium quality with high concentration of apigenin malonyl glucoside, caffeic acid, ferulic acid and kaempferolo-3-*O*-rutinoside

(**Figure 3**). Moreover, purslane grown under monochromatic blue or red LED lighting was positioned in the lower left quadrant, characterized overall by higher chlorogenic acid, rutin and total polyphenols (**Figure 3**).

3.6. Principal Component Analysis of Growth Parameters, Mineral Profile, Nutritional and Functional Traits of Microgreen Species Grown under Variable Bandwidths

To obtain an in-depth overview of productivity, mineral profile, nutritional and functional traits of the four microgreens genotypes as modulated by variable spectral bandwidths, a second PCA was performed on fresh yield, dry matter, mineral composition (nitrate, P, K, Ca, Mg, S and Na), carotenoid composition (lutein and *β*-carotene) and total phenolics obtained and discussed above (**Figure 4**).

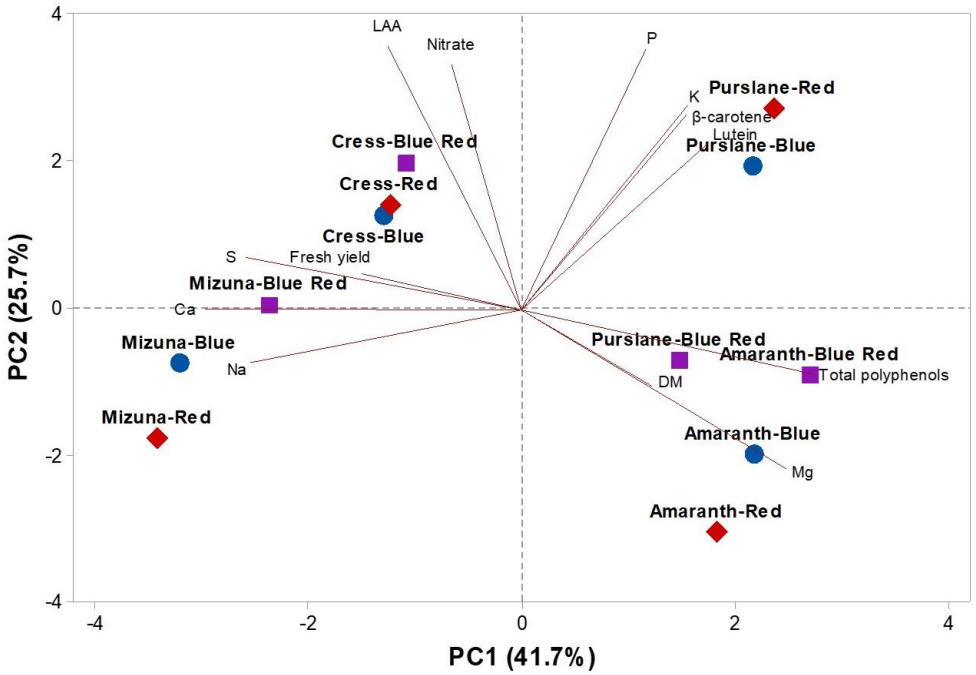

Figure 4. Principal component loading plot and scores of principal component analysis (PCA) fresh yield and dry matter content, mineral concentrations (nitrate, P, K, S, Ca, Mg and Na), lipophilic antioxidant activity (LAA), lutein, *β*-carotene and total phenolics concentrations in four microgreens genotypes as modulated by variable spectral bandwidths in a controlled growth environment.

The first three principal components (PCs) were related with eigen values > 1 and explained 85.5% of the total variance, with PC1, PC2, and PC3 accounting for 41.7%, 25.7% and 18.1%, respectively (**Supplementary Table S2**).

Supplementary Table 2. Eigen values, relative and cumulative proportion of total variance, and correlation coefficients for growth parameters, mineral profile, nutritional and functional traits in four microgreens genotypes as modulated by variable spectral bandwidths with respect to the three principal components.

Principal components	**PC1**	**PC2**	**PC3**
Eigen value	5.4	3.3	2.3
Percentage of variance	41.7	25.7	18.1
Cumulative variance	41.7	67.4	85.5
Eigen vectors [a]			
Ca	**-0.971**	0.004	-0.034
Total polyphenols	**0.900**	-0.199	0.317
S	**-0.846**	0.164	-0.483
Na	**-0.830**	-0.162	0.472
Mg	**0.812**	-0.492	0.129
LAA	-0.410	**0.818**	0.041
P	0.381	**0.806**	0.163
Nitrate	-0.215	**0.761**	-0.499
K	0.510	**0.634**	0.220
β-carotene	0.506	**0.604**	0.106
DM	0.401	-0.237	**-0.854**
Fresh yield	-0.489	0.111	**0.845**
Lutein	**0.600**	0.518	-0.004

[a]Boldface factor loadings are considered highly weighed

[b] LAA, Lipophylic antioxidant activity; DM, dry matter.

Furthermore, the loading matrix indicates the correlations among the examined quanti-qualitative traits. In our study, we discerned four groups of positively correlated variables (**Figure 4**): i) the group in the upper left quadrant comprising nitrate and lipophilic antioxidant activity, ii) the group in the upper right quadrant comprising the two carotenoids, P and K, iii) the group clustered in the lower right quadrant comprising total polyphenols

and Mg and finally iv) the group in the lower left quadrant comprising fresh yield and most of the mineral composition (S, Ca and Na; **Figure 4**).

The effectiveness of PCA in interpreting genotype differences across multiple nutritional and functional quality characters in response to several pre-harvest factors (e.g., light conditions, nutrient solution management and biofortification) has been previously demonstrated (Colonna et al., 2016; Cardarelli et al., 2017; Rouphael et al., 2017b, 2017a; El-Nakhel et al., 2019; Kyriacou et al., 2019a). This was also the case in the current experiment, since the score plot of the PCA highlighted crucial information on the nutritional and functional quality of the tested microgreens genotypes exposed to variable bandwidths. The PCA clearly divided the four tested microgreens along PC1 with *Brassicaceae* microgreens (cress and mizuna) on the negative side and amaranth and purslane microgreens on the positive side (**Figure 4**). Accordingly, *Brassicaceae* microgreens distinguished for higher fresh yield, nitrate and mineral profile (S, Ca and contents); whereas the amaranth and purslane were superior in target lipophilic antioxidant molecules as well as in total polyphenols (**Figure 4**). Particularly, the purslane grown under monochromatic red LED lighting, positioned in the upper right quadrant of the PCA score plot, delivered premium quality and high concentration of lipophilic antioxidants such as lutien and β-carotene and P content (**Figure 4**). Moreover, cress grown under monochromatic and dichromatic red and blue LED lighting was positioned in the upper left quadrant, characterized overall by higher lipophilic antioxidant activity and nitrate content. Finally, the lower left quadrant depicted treatments (mizuna grown under red/blue/blue-red LED lighting) characterized by high fresh yield, Ca and S contents (**Figure 4**). The PCA performed in the present experiment configured an integrated view of fresh yield and quality traits quantified by ion chromatography, HPLC-DAD and UHPLC-Q-Orbitrap HRMS. Thus it enabled the interpretation of variation patterns in these traits with respect to the microgreens genotypes and the studied select spectral bandwidths.

4. Conclusions

Targeted modulation of microgreens secondary metabolism through select spectral bandwidths is presently assessed as a tool to produce phytochemically-enriched microgreens of high functional quality and nutritive value. Growth and yield parameters dependent on

primary metabolism were optimized under dichromatic blue-red light. Nitrogen assimilation mediated by nitrate and nitrite reduction was hampered under monochromatic blue and red lights but was more efficiently promoted by dichromatic blue-red light. Therefore, use of monochromatic blue or red light as opposed to combined blue-red bandwidths may result in higher accumulation of nitrate in microgreens. Spectral effects seemed less consistent with respect to microgreens' mineral composition, variation in which was primarily genotypic. Concentrations of key carotenoids lutein and β-carotene and the lipophilic antioxidant capacity of microgreens were favoured by blue-red light. Purslane however diverged from this response pattern, highlighting the underlying genotype specificity of these responses that requires additional investigation. Analysis of polyphenols by Orbitrap LC-MS/MS revealed substantial genotypic differences with respect to composition. The general response across species was a decrease in individual polyphenolic constituents, particularly flavonol glycosides, and total polyphenols under blue-red light. The current work highlights how select genetic background combined with effective light management might facilitate production of microgreens with superior functional quality.

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Chapter 4: Phenolic Constitution, Phytochemical and Macronutrient Content in Three Species of Microgreens as Modulated by Natural Fiber and Synthetic Substrates

antioxidants

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Article

Phenolic Constitution, Phytochemical and Macronutrient Content in Three Species of Microgreens as Modulated by Natural Fiber and Synthetic Substrates

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Phenolic Constitution, Phytochemical and Macronutrient Content in Three Species of Microgreens as Modulated by Natural Fiber and Synthetic Substrates

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Abstract

The present study examined the modulatory effects of natural fiber substrates (agave fiber, coconut fiber and peat moss) and synthetic alternatives (capillary mat and cellulose sponge) on the nutritive and phytochemical composition of select microgreens species (coriander, kohlrabi and pak choi) grown in a controlled environment. Polyphenols were analyzed by UHPLC-Q-Orbitrap-HRMS, major carotenoids by HPLC-DAD, and macro-minerals by ion chromatography. Microgreens grown on peat moss had outstanding fresh and dry yield but low dry matter content. Natural fiber substrates increased nitrate and overall macro-mineral concentrations in microgreens compared to synthetic substrates. The concentrations of chlorophylls, carotenoids and ascorbate were influenced primarily by species. On the contrary, variability in polyphenols content was wider between substrates than species. Out of twenty phenolic compounds identified, chlorogenic acid and quercetin-3-O-rutinoside were most abundant. Hydroxycinnamic acids and their derivatives accounted for 49.8% of mean phenolic content across species, flavonol glycosides for 48.4% and flavone glycosides for 1.8%. Peat moss provided optimal physicochemical conditions that enhanced microgreens growth rate and biomass production at the expense of phenolic content. In this respect, the application of controlled stress (eustress) on microgreens growing on peat moss warrants investigation as a means of enhancing phytochemical composition without substantial compromise in crop performance and production turnover. Finally, nitrate

deprivation practices should be considered for microgreens grown on natural fiber substrates in order to minimize consumer exposure to nitrate.

Keywords: *agave fiber; capillary mat; cellulose sponge; coriander; carotenoids; flavonoids; kohlrabi; nitrate; Orbitrap LC-MS/MS; pak choi; phenolic compounds*

1. Introduction

Over the past three decades, several researchers have tried to boost the content of vegetables in phytochemicals (e.g., ascorbate, carotenoids, glucosinolates, polyamines, polyphenols) through plant breeding and biotechnology, but so far, they met limited commercial success due to safety issues (Poiroux-Gonord et al., 2010). Therefore, searching for nutrient-dense vegetables through the manipulation of phytochemicals by environmental (air temperature, light quality, intensity and photoperiod) and innovative crop management practices (growing media, nutrition and biofortification) represents a promising and balanced approach between safety, cost and effectiveness (Kyriacou and Rouphael, 2018; Rouphael et al., 2018). Microgreens (i.e., edible seedlings of herbs, grains and vegetables), also known as vegetable confetti, are an emerging class of specialty crop that have gained increasing popularity among consumers, urban farmers, food technologists and nutritionists due to their fortified phytochemical composition, accumulated in the two fully developed cotyledons and the first true leaves, compared to their mature counterparts (Xiao et al., 2012, 2015; Di Gioia et al., 2015; Pinto et al., 2015; Choe et al., 2018; Lenzi et al., 2019). In addition to their potential nutritional and functional benefits, microgreens production presents the following advantages: (i) short cultivation cycle, (ii) all-year round production, (iii), ease of cultivation, (iv) suitability for indoor farming technology, (v) high potential returns/profitability for producers with an estimated value of 30–50 $ per pound and (vi) higher sustainability compared to growing mature herbs and vegetables, offering a small footprint in terms of space, water and fertilizers (Kyriacou et al., 2016a, 2017; Choe et al., 2018). Moreover, the brief production cycle combined with rigorous potential returns for farmers makes microgreens a prominent controlled environment candidate crop (Choe et al., 2018).

Although several studies revealed that variation in microgreens' content of bioactive compounds is based on several pre-harvest factors such as genetic material (i.e., species),

conditions of cultivation and light parameters (i.e., spectral quality and intensity), additional variables have also been implicated in shaping microgreens' nutritive and phytochemical composition, including nutrition/biofortification and choice of growth medium (Xiao et al., 2012, 2019; Brazaitytė et al., 2015; Kyriacou et al., 2016a, 2019a, 2019c; Samuolienė et al., 2016; Craver et al., 2017; Samuolienė et al., 2017; Vaštakaite et al., 2017; Alrifai et al., 2019; De la Fuente et al., 2019). Notwithstanding the short crop cycle, special attention must be placed on the selection of growth media for microgreens, which represents one of the most important factors in the production process influencing microgreens quality (Di Gioia et al., 2017). Among common growing media used to produce microgreens, peat-based media come first, followed by coconut coir and synthetic fibrous media (Kyriacou et al., 2016a). Recently, natural fiber-based media such as burlap, jute, cotton, cellulose pulp, kenaf and hemp fibers have gained increasing popularity in the microgreens industry, since they represent natural, sustainable and cheap alternatives for microgreens production (Kyriacou et al., 2016a; Di Gioia et al., 2017). However, few studies have examined the effects different types or combinations of media have on the yield of variable microgreens genotypes (Muchjajib et al., 2015; Di Gioia et al., 2017). Muchjajib et al. (2015) demonstrated that the 1:1 mixture of coconut coir dust and peat provided the highest yield for spinach microgreens, while the 1:1 mix of coconut coir dust with sugarcane filter cake exhibited the maximum yield for radish, mustard, krathin and kangkong microgreens. Additionally, Di Gioia et al. (2017) and co-workers demonstrated that organic (peat) and recycled fibrous materials (textile and jute-kenaf fibers) supported a fresh yield of rapini (*Brassica rapa* L.) higher by 15% to that on the synthetic fibrous material Sure-to-Grow®, which is marketed as a substrate consisting of plastic fibers with high water retention capacity. The same authors reported that peat-grown rapini microgreens had the highest population of *Escherichia coli* and *Enterobacteriaceae*, which was not detected on *B. rapa* microgreens grown on other substrates. However, scientific information on phytochemical profiles, and how these bioactive secondary metabolites respond to organic, synthetic and novel by-product substrates in emerging microgreens like coriander, kohlrabi and pak choi is completely missing.

Microgreens are currently considered among the five most profitable crops globally, along with mushrooms, ginseng, saffron and goji berries. Therefore, developing species-

specific growth media to support year-round production and to enhance valuable antioxidant components is affordable and of utmost importance for the microgreens industry; particularly as the latter is characterized by high investment in technology (e.g., lighting and growth substrates) driven by the necessity to cultivate novel, highly fortified microgreen species. Considering the above considerations, the present study aimed (i) to characterize and elucidate the modulatory effects of natural fiber substrates (agave fiber, coconut fiber and peat moss) and synthetic substrates (capillary mat and cellulose sponge) on the nutritional and phytochemical composition (minerals, nitrate, chlorophylls, target carotenoids, ascorbate and polyphenols) of select microgreens (coriander, kohlrabi and pak choi), and (ii) to appraise possible clustering patterns underscoring microgreens composition and substrates physico-chemical characteristics.

2. Materials and Methods

2.1. Reagents and Standards Preparation

The reagents methanol and formic acid (LC-MS grade) were purchased from Merck KGaA (Darmstadt, Germany); *β*-carotene, chicoric acid, chlorogenic acid, caffeic acid, catechin, epicatechin, ferulic acid, lutein, quercetin-3-*O*-glucoside, rosmarinic acid, rutin and vitexin standards were purchased from Sigma (St. Louis, MO, USA) and 3,5-di-*O*-caffeoyl quinic acid, kaempferol-7-*O*-glucoside, kaempferol-3-*O*-rutinoside and quercetin-3-*O*-galactoside from Extrasynthese (Genay, France). A Milli-Q Gradient A10 water purification system (Merck Millipore, Darmstadt, Germany) was used to produce ultrapure water. The standards were 98% pure, prepared with methanol to form 1 mg mL^{-1} initial stock solutions, while for lutein and *β*-carotene, chloroform was used to prepare the stocks of 1 mg mL^{-1} as well. Then, in order to acquire standard calibration curves of 0.01–5.0 mg L^{-1} span, individual standard stock solutions were combined to prepare multiple standards stock solutions by applying further dilutions made with methanol.

2.2. Plant Material and Climate Chamber Conditions

Three microgreen species, kohlrabi (*Brassica oleracea* L. var. *gongylodes*; Purple Vienna, Condor Seed Production, Yuma, AZ, USA), pack choi (*Brassica rapa* L. subsp. *chinensis*; Red Wizard F1, CN Seeds Ltd., Pymoor, Ely, Cambrigeshire, UK) and coriander

(*Coriandrum sativum* L.; Micro Splits, CN Seeds Ltd., Pymoor, Ely, Cambrigeshire, UK), were sown in 5 different substrates: agave fiber (Sisal Fibre, Imola, Italy), capillary mat (Aquamat capillary matting, Premier Netting, Norfolk, UK), cellulose sponge (Spontex SAS, Colombes, France), coconut fiber (Sisal Fibre, Imola, Italy) and peat moss (Floragard, Oldenburg, Deutschland). Sowing density was 60,000, 63,000 and 46,000 seeds m^{-2} for kohlrabi, pack choi and coriander, respectively. Hundred-seed weight was 0.320, 0.240 and 0.684 g for kohlrabi, pack choi and coriander, respectively.

Experiments were carried out in a climate chamber (Panasonic MIR-554, Gunma, Japan) at the Agricultural Research Institute (ARI), Nicosia, Cyprus, using Light Emitting Diode (LED) panel units (K5 Series XL750, Kind LED, Santa Rosa, CA, USA) procuring a photosynthetic photon flux density (PPFD) of 300 ±10 µmol m^{-2} s^{-1} and a spectral composition matching the optimal absorption spectrum of photosynthesis. Seed germination occurred in darkness at 24 °C and 100% relative humidity. Day/night temperatures during the growth cycle were set at 22/18 ± 1 °C with a 12 h photoperiod and a relative air humidity of 65%/75% ± 5%. These levels of relative humidity ensured that the development of potentially harmful mycotoxins-producing molds was not observed in our experiments. The substrates were placed in plastic trays (14 × 19 × 6 cm: W × L × D). Fertigation was applied everyday manually by means of a laboratory beaker. The nutrient solution corresponded to a quarter-strength modified Hoagland formulation (2.0 mM nitrate, 0.25 mM sulfur, 0.20 mM phosphorus, 0.62 mM potassium, 0.75 mM calcium, 0.17 mM magnesium, 0.25 mM ammonium, 20 µM iron, 9 µM manganese,0.3 µM cupper, 1.6 µM zinc, 20 µM boron, and 0.3 µM molybdenum), with an electrical conductivity (EC) and pH of 0.4 ± 0.1 dS m^{-1} and 6 ± 0.2, respectively. Each treatment was replicated three times, and the substrate trays were positioned randomly in the climate chamber. Daily rotation of the trays was performed to ensure homogenous light and humidity across the shelf surface.

2.3. Growing Substrates Physicochemical Characterization

The physical properties of the five growing substrates, such as bulk density, total pore space, water holding capacity and air capacity, were appraised according to Di Gioia et al. (2017). For the assessment of chemical properties, substrate samples were dried to a constant weight at 105 °C. The EC and pH of the different substrates were determined in 1:2.5 substrate:water suspensions stirred overnight before measurements were performed. A

benchtop electrical conductivity meter (SevenMulti, Metler-Toledo GmbH, Greifensee, Switzerland) and an electrochemical pH meter (SevenMulti; Mettler-Toledo GmbH, Schwerzenbach, Switzerland) were used for these measurements.

For the mineral content analysis of the substrates, 500 mg of each substate was suspended in 50 mL ultrapure water and shook in an orbital lab shaker (KS125 basic, IKA, Staufen, Germany) for 10 min. Later on, the water extracts were analyzed to determine the concentration of NO_3^-, P, K^+, Ca^{2+}, Mg^{2+}, S, NH_4^+, Na^+ and Cl^- by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) with a conductivity detector. All physical and chemical analyzes were performed in triplicate, and the results are listed in **Table 1**.

2.4. Harvesting Schedule, Sampling, Growth Analysis and Colorimeter Measurement

Shortly before harvesting, the microgreens' canopy color lightness (L*) was measured at six different points on each plastic tray using an 8 mm-aperture Minolta CR-400 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan). All microgreens were harvested as soon as the second true leaf emerged, by cutting at the substrate level. The harvested material was weighed to determine the fresh weight, expressed in kg (fw) m^{-2}. Directly after, 10 g of fresh microgreens were instantly stored in liquid nitrogen and then stored at −80 °C prior to liophilization in a freeze drier (Christ, Alpha 1-4, Osterode, Germany).

Microgreens' dry weight (dw) was measured on an analytical balance (XT120A; Precisa Gravimetrics, Dietikon, Switzerland) after desiccation of the remaining material in a forced-air oven at 65 °C until reaching constant weight. Dry matter (DM) content was also calculated and expressed as a percentage of microgreens' fresh mass. The dry material (microgreens leaves and stems) was ground in a Wiley Mill to pass through an 841-microns screen and used for chemical analyses.

2.5. Mineral Analysis, Nitrate, Total Chlorophyll and Total Ascorbic Acid

Nitrate, macro-minerals and sodium concentrations were determined by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) coupled to a conductivity detector, as described in (Rouphael et al., 2017a). The results were expressed in g kg^{-1} dw, except for nitrate that was converted to mg kg^{-1} fw based on each sample's dw. Total chlorophyll content (mg kg^{-1} fw) was extracted by grinding 200 mg fw of microgreens in

80% ammoniacal acetone using a mortar and pestle; then the extract was centrifuged for 3 min at 3000g. The supernatant absorbance was read at 647 and 664 nm through a Hach DR 2000 spectrophotometer (Hach Co., Loveland, Colorado, USA) to determine the content of chlorophyll a and b, respectively, and their sum was taken as the total chlorophyll content (Lichtenthaler and Buschmann, 2001).

As reported by Kampfenkel et al. (1995), the sum of ascorbic and dehydroascorbic acids defined the total ascorbic acid concentration assessed by UV–Vis spectrophotometry (Hach DR 2000; Hach Co., Loveland, CO, USA). The quantification was carried out at 525 nm against an ascorbic acid (AA) standard calibration curve (5–100 μmol mL^{-1}) and expressed in mg AA kg^{-1} fw.

2.6. Carotenoids and Polyphenols Extraction and Quantification

Lutein and *β*-carotene were extracted from lyophilized samples and separated on a Shimadzu HPLC Model LC 10 (Shimadzu, Osaka, Japan) using a reverse phase 250 × 4.6 mm, 5 μm Gemini C18 column (Phenomenex, Torrance, CA, USA) according to the method described by Kim et al. (2008) and the modifications introduced by (Kyriacou et al., 2019a). Quantification was performed against calibration curves built with lutein and *β*-carotene external standards (5–100 μg mL^{-1}) and results were expressed in mg kg^{-1} dw. The levels of carotenoids were calculated using the regression equation $y = 1.163x - 994$ ($r^2 = 0.992$) for *β*-carotene and $y = 1.053x - 0.651$ ($r^2 = 0.990$) for lutein. The limit of quantification (LOQ) was calculated for each standard and was determined as the lowest injected amount which could be reproducibly quantified (RSD ≤ 3%). The LOQ value was 0.25 ppm for both carotene and lutein.

Polyphenols were also extracted from lyophilized samples and separated on a UHPLC system (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Kinetex 1.7 μm Biphenyl (100 × 2.1 mm) column (Phenomenex, Torrance, CA, USA) according to the conditions described by Llorach et al. (2008) and Kyriacou et al. (2019a). Mass spectrometry analysis was performed on a Q Exactive Orbitrap LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA). Acquisition of polyphenolic compounds was carried out on parallel reaction monitoring (PRM). This modality of acquisition allows a targeted MS/MS analysis using the mass inclusion list and expected retention times of the target analytes, with a 30 s time window, with the Orbitrap spectrometer operating in negative

mode at 17,500 FWHM (*m/z* 200). The AGC target was set to 2e5, with the maximum injection time of 20 ms. The precursor ions in the inclusion list were filtered by the quadrupole at an isolation window of *m/z* 2 and fragmented in an HCD collision cell set at 30 Kv. A mass tolerance of 5 ppm was employed. The instrument calibration was checked daily using a reference standard mixture obtained from Thermo Fisher Scientific.

2.7. Statistics

Experimental data were subjected to bifactorial (microgreens species × substrate) analysis of variance using SPSS 20 software package (IBM, Armonk, NY, USA). Treatment means were separated by Duncan's Multiple Range Test performed at $p \leq 0.05$. Yield and compositional characteristics of microgreens were subjected to principal component analysis (PCA) to explore relationships among variables and to compare the collective effects of substrates on these traits.

3. Results and Discussion

3.1. Substrate Physicochemical Properties

The five media presently evaluated as substrates for microgreens presented significant variation in physicochemical constitution (**Table 1**). The mechanical properties of a substrate, particularly its porosity, are associated with its bulk density (BD), which also reflects on the transport cost for its distribution (Di Gioia et al., 2017). The BD ranged from the lightest cellulose sponge (39.6 kg m^{-3}) to the heaviest capillary mat (240.7 kg m^{-3}), whereas peat moss, agave fiber, and coconut fiber had moderate BD (112.4–147.8 kg m^{-3}). Nonetheless, all media registered a BD below 400 kg m^{-3}, considered the maximum acceptable value according to the horticultural media inventory by Abad et al. (2011). Total pore space (TPS), which is the sum of a medium's water-holding and air-holding capacities (WC and AC, respectively) was lowest in capillary mat (86.4% *v/v*) and highest in agave fiber (95.6 % *v/v*), yet all substrates were within the optimal TSP range (>85 %; [29]). However, substrates differed markedly in the type of porosity as indicated by differences in WC and AC (**Table 1**). Peat moss had the lowest WC (58.8 %) and inversely the highest AC (28.7 %), reflecting its low content of micropores and high content of macropores. The low AC of cellulose sponge, capillary mat and coconut fiber reflects their high relative content

of micropores that provides a less favorable environment for root function. The above results carry important implications for the behavior of the tested substrates particularly with regard to the frequency and volume of irrigation. An ideal growing medium must combine physical properties sustaining a favorable balance between aeration and water holding during and between irrigation events so as to avoid water potential extremes and hypoxia conditions in the root zone (Barrett et al., 2016). The synthetic substrates of the capillary mat and cellulose sponge were restrained by low AC and their use for microgreen cultivation would require reduced frequency and/or volume of irrigation in order to sustain adequate root aeration (Savvas, 2007). Peat moss, on the other hand, showed higher AC but lower WC than the rest of the natural fiber substrates and would thus require more frequent controlled irrigation. It must be noted, however, that the generally optimal physical properties of peat may vary depending on the material used for its production, with less decomposed peats demonstrating higher WC than older and more decomposed material (Schmilewski, 2008). Further to peat, agave fiber combined near optimal physical properties of AC and WC, with the latter being only slightly supra-optimal.

Peat moss and agave fiber also presented the lowest electrical conductivity (EC; 282 and 254 μS cm^{-1}, respectively) whereas capillary mat had the highest (1258 mS cm^{-1}; **Table 1**). The EC of coconut fiber (879 μS cm^{-1}) was also notably high, derived likely from the processing of raw material with saline water in coastal areas of coconut production [30]. The variably high EC of coconut fiber draws additional cost for salt-leaching treatments of this otherwise cheap and renewable material, comparable to peat, before being used as a substrate for salt-sensitive crops like microgreens. Substrates of inherently high salt concentration have been shown to impair seed germination and seedling growth, although the severity of deleterious effects is species-dependent (Sánchez-Monedero et al., 2004).

Coconut fiber, peat moss and capillary mat were all mildly acidic (pH 5.34–5.71) and their pH may be considered optimal for facilitating the availability of nutrients supplied through fertigation (**Table 1**; Abad et al., 2011). Agave fiber was the most acidic medium (pH 4.90) and cellulose sponge is outstandingly the most alkaline (pH 8.49). The pH of agave fiber may be easily adjusted through the nutrient solution, whereas cellulose sponge may require an acidification pretreatment. Residual nitrate was lowest in peat moss and capillary mat (0.24 and 0.54 g kg^{-1} dw, respectively) and highest in agave fiber (9.07 g kg^{-1} dw).

Ammonium residue was highest in capillary mat (3.66 g kg^{-1} dw) and lowest in cellulose sponge and peat moss (0.20 and 0.42 g kg^{-1} dw). Cellulose sponge and capillary mat were deficient in phosphorous (0.01 and 0.07 g kg^{-1} dw, respectively), which was most abundant in coconut fiber (1.72 g kg^{-1} dw). Cellulose sponge was also very low in potassium content (1.93 g kg^{-1} dw), which was exceptionally high in coconut fiber (81.41 g kg^{-1} dw). Peat moss was the substrate highest in calcium content (30.34 g kg^{-1} dw) and capillary mat the lowest (3.34 g kg^{-1} sw). Relatively limited variation was observed for magnesium content which was higher in cellulose sponge (12.23 g kg^{-1} dw) than the rest media (2.66–5.73 g kg^{-1} dw). Sulphur was lowest in agave fiber (0.98 g kg^{-1} dw) and highest in capillary mat (9.15 g kg^{-1} dw). While the deficiencies in macronutrients described above may be remedied through the nutrient solution, the concentrations of sodium and chloride are critical and require close monitoring to avert salt stress on tender microgreens (Shannon and Grieve, 1998). Peat moss was the substrate found to be lowest in both sodium (3.89 g kg^{-1} dw) and chloride (2.05 g kg^{-1} dw) content. Sodium content was highest in capillary mat (21.73 g kg^{-1} dw), while chloride was highest in coconut fiber (77.62 g kg^{-1} dw) and cellulose sponge (37.65 g kg^{-1} dw). Based on the overall physicochemical profile of the appraised substrates, peat moss and agave fiber present the most optimized environment for microgreens cultivation.

Table 1. Physicochemical characterization of natural fiber and synthetic substrates used for coriander, kohlrabi and pak choi microgreens production in a controlled environment. All data are expressed as mean ± standard error, $n = 3$.

Physicochemical Parameters	Substrate					Significance
	Agave Fiber	Capillary Mat	Cellulose Sponge	Coconut Fiber	Peat Moss	
Bulk density (kg m^{-3})	144.2 ± 4.49^{b}	240.7 ± 6.24^{a}	39.6 ± 0.23^{d}	112.4 ± 0.19^{c}	147.8 ± 1.15^{b}	***
Total pore space (% *v/v*)	95.6 ± 0.28^{a}	86.4 ± 0.52^{d}	93.8 ± 0.42^{b}	90.8 ± 0.16^{c}	87.4 ± 0.16^{d}	***
Water-holding capacity (% *v/v*)	76.6 ± 0.3^{b}	71.1 ± 0.5^{d}	80.2 ± 0.58^{a}	74 ± 0.12^{c}	58.8 ± 0.27^{e}	***
Air capacity (% *v/v*)	19 ± 0.25^{b}	15.3 ± 0.21^{d}	13.7 ± 0.61^{e}	16.8 ± 0.07^{c}	28.7 ± 0.27^{a}	***
EC (µS cm^{-1})	254 ± 0.50^{e}	1258 ± 8.00^{a}	702 ± 0.50^{c}	879 ± 1.00^{b}	282 ± 1.50^{d}	***
pH	4.90 ± 0.005^{e}	5.71 ± 0.010^{b}	8.49 ± 0.010^{a}	5.34 ± 0.005^{d}	5.48 ± 0.005^{c}	***
NO_3 (g kg^{-1} dw)	9.07 ± 0.21^{a}	0.54 ± 0.11^{d}	1.16 ± 0.06^{c}	2.63 ± 0.18^{b}	0.24 ± 0.05^{d}	***
P (g kg^{-1} dw)	0.67 ± 0.03^{c}	0.07 ± 0.00^{d}	0.01 ± 0.00^{e}	1.72 ± 0.00^{a}	1.04 ± 0.01^{b}	***
K (g kg^{-1} dw)	15.00 ± 0.32^{b}	13.39 ± 0.03^{bc}	1.93 ± 0.92^{d}	81.41 ± 1.83^{a}	12.10 ± 0.88^{c}	***
Ca (g kg^{-1} dw)	10.66 ± 0.02^{c}	3.44 ± 0.26^{e}	9.89 ± 0.09^{d}	16.67 ± 0.29^{b}	30.34 ± 0.19^{a}	***
Mg (g kg^{-1} dw)	4.92 ± 0.95^{b}	2.75 ± 0.74^{b}	12.23 ± 0.59^{a}	5.73 ± 0.33^{b}	2.66 ± 1.17^{b}	**
S (g kg^{-1} dw)	0.98 ± 0.14^{d}	9.15 ± 0.00^{a}	1.72 ± 0.01^{c}	1.10 ± 0.16^{d}	6.09 ± 0.09^{b}	***
NH_4 (g kg^{-1} dw)	2.12 ± 0.06^{b}	3.66 ± 0.05^{a}	0.20 ± 0.16^{c}	1.68 ± 0.05^{b}	0.42 ± 0.16^{c}	***
Na (g kg^{-1} dw)	6.72 ± 0.08^{d}	21.73 ± 0.16^{a}	12.18 ± 0.37^{c}	19.80 ± 0.11^{b}	3.89 ± 0.29^{e}	***
Cl (g kg^{-1} dw)	9.58 ± 0.05^{c}	6.42 ± 0.77^{d}	37.65 ± 0.40^{b}	77.52 ± 1.09^{a}	2.05 ± 0.06^{e}	***

ns,**, *** Nonsignificant or significant at $p \leq 0.01$, and 0.001, respectively. Different superscript letters (a–e) within each row indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.2. Fresh Biomass Yield, Dry Matter Content, Canopy Height and Color

Species germination was progressively slower in the order pak choi, kohlrabi and coriander, as indicated by the intercepts and slopes of **Figure 1A–C.** Despite late germination, coriander microgreens exhibited faster growth than the other two species in the 2–4-day period immediately after emergence. The germination of coriander and pak choi was slowest on synthetic capillary mat, while for kohlrabi the slowest germination was on cellulose sponge. Considering the brief crop cycle of microgreens, the cost of controlled growth conditions and the demand for high turnover, a lag period of 1–2 days in the germination process may constitute an important setback associated with the above synthetic substrates (Kyriacou et al., 2016a); which might in fact derive from their relatively low AC properties (Savvas, 2007). Moreover, all species demonstrated the tallest canopy throughout the growth period when grown on peat moss and the shortest when grown on capillary mat, except for coriander, which was shortest on coconut fiber (**Figure 1A–C**). Moreover, maximum canopy height was reached about two days earlier on peat moss compared to the rest of the substrates. It is thus apparent that the growth of all microgreen species availed of the optimal air-moisture-salinity conditions provided by peat moss and to a lesser extent by agave and coconut fibers. It is also evident that the inherently high EC of the synthetic substrates as well as that of coconut fiber can set back the growth of microgreens and prolong the crop cycle, especially of salt-sensitive species such as coriander (Sánchez-Monedero et al., 2004; Okkaoğlu et al., 2015).

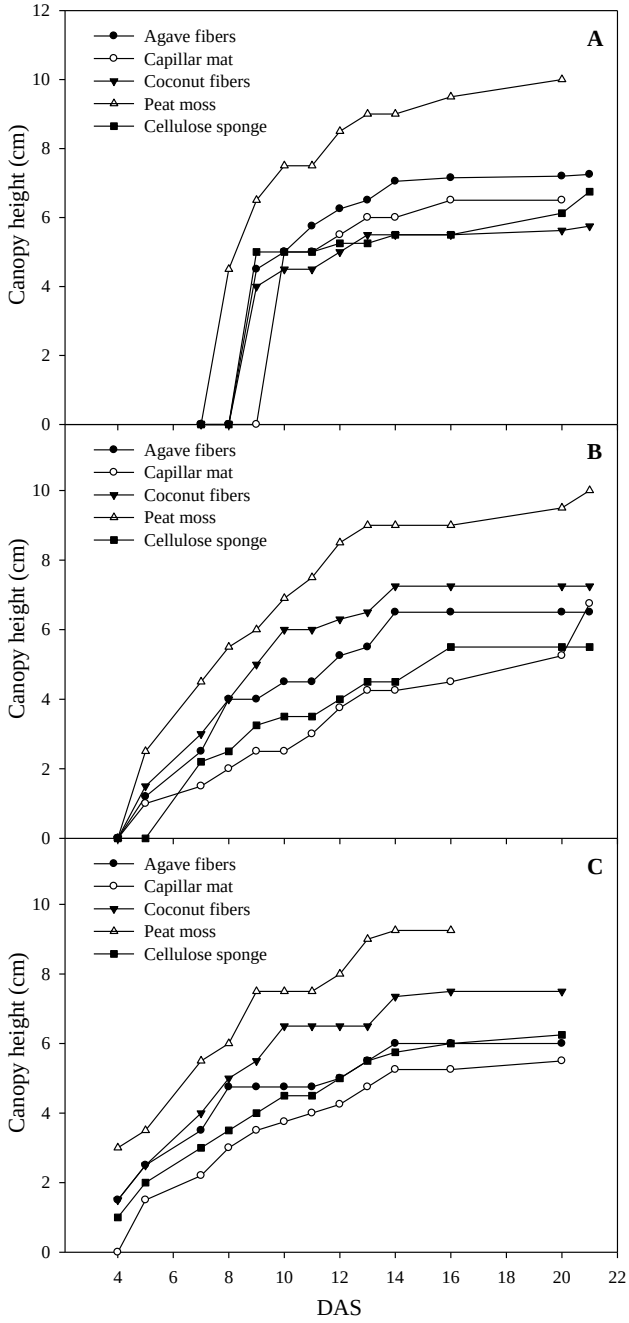

Figure 1. Canopy height of coriander (A), kohlrabi (B) and pak choi (C) microgreens while growing on natural fiber and synthetic substrates under controlled environment. DAS: days after sowing. All data are expressed as mean ± standard error, $n = 3$.

The mean fresh yield obtained across species (1.66 ± 0.30 kg fw m^{-2}) compared favorably to the yields reported for several microgreens' species by previous workers (Bulgari et al., 2017; Di Gioia et al., 2017). Moreover, the current results indicate that the mean fresh yield of pak choi and kohlrabi microgreens was 28.4% higher than that of coriander, regardless of substrate (**Table 2**), which underscores previous reports of starkly differentiated growth rates and biomass production in different species of microgreens (Kyriacou et al., 2016a). Regardless of interspecific differences in yield, microgreens of all species yielded outstandingly when grown on peat moss, amounting to a 55.1% increase compared to the other four substrates.

Table 2. Fresh and dry yield, dry matter content and canopy lightness (L*) of coriander, kohlrabi and pak choi substrates grown on natural fiber and synthetic substrates under a controlled environment. All data are expressed as mean ± standard error, $n = 3$.

Source of Variance	Yield (kg fw m^{-2})		Dry Weight (g m^{-2})		Dry Matter (%)		L*	
Microgreens Species (M)								
Coriander	1.31	± 0.14^{b}	169.03	± 11.06	13.59	± 0.52^{a}	33.68	± 0.30^{a}
Kohlrabi	1.80	± 0.24^{a}	182.89	± 17.63	10.71	± 0.37^{b}	28.52	± 0.26^{b}
Pak choi	1.86	± 0.20^{a}	172.75	± 8.18	10.12	± 0.64^{b}	23.87	± 0.27^{c}
Substrate (S)								
Agave fiber	1.18	± 0.05^{c}	143.46	± 8.92^{c}	12.16	± 0.42^{b}	29.57	± 1.39^{a}
Capillary mat	1.18	± 0.12^{c}	134.73	± 7.17^{c}	11.95	± 0.68^{b}	27.98	± 1.26^{c}
Cellulose sponge	1.31	± 0.06^{c}	172.81	± 5.22^{b}	13.30	± 0.52^{a}	28.27	± 1.57^{bc}
Coconut fiber	1.65	± 0.18^{b}	178.71	± 9.43^{b}	11.54	± 0.94^{b}	28.72	± 1.36^{abc}
Peat moss	2.96	± 0.19^{a}	244.75	± 17.17^{a}	8.43	± 0.57^{c}	28.91	± 1.64^{ab}
M × S								
Coriander × Agave fiber	1.28	± 0.04^{ef}	175.33	± 8.20^{cd}	13.66	± 0.39^{b}	34.49	± 0.19
Coriander × Capillary mat	0.80	± 0.08^{g}	111.04	± 8.59^{g}	13.91	± 0.39^{ab}	32.55	± 0.90
Coriander × Cellulose sponge	1.16	± 0.12^{efg}	174.67	± 14.95^{cd}	15.11	± 0.32^{a}	33.63	± 0.49
Coriander × Coconut fiber	1.02	± 0.02^{fg}	153.66	± 5.15^{def}	15.12	± 0.74^{a}	33.27	± 0.05
Coriander × Peat moss	2.27	± 0.10^{b}	230.45	± 13.00^{b}	10.12	± 0.14^{ef}	34.49	± 0.82
Kohlrabi × Agave fiber	1.08	± 0.04^{fg}	121.70	± 4.95^{fg}	11.29	± 0.12^{de}	29.16	± 0.48
Kohlrabi × Capillary mat	1.20	± 0.02^{ef}	144.69	± 8.70^{def}	12.07	± 0.85^{cd}	27.21	± 0.31
Kohlrabi × Cellulose sponge	1.41	± 0.03^{ef}	164.00	± 3.24^{de}	11.65	± 0.10^{d}	28.32	± 0.49
Kohlrabi × Coconut fiber	1.86	± 0.23^{cd}	180.27	± 20.61^{cd}	9.72	± 0.42^{f}	28.93	± 0.59
Kohlrabi × Peat moss	3.43	± 0.18^{a}	303.77	± 15.78^{a}	8.84	± 0.05^{f}	28.98	± 0.47
Pak choi × Agave fiber	1.17	± 0.13^{efg}	133.33	± 8.20^{efg}	11.54	± 0.50^{d}	25.05	± 0.68
Pak choi × Capillary mat	1.53	± 0.17^{de}	148.44	± 6.60^{def}	9.86	± 0.76^{f}	24.19	± 0.46
Pak choi × Cellulose sponge	1.37	± 0.08^{ef}	179.77	± 5.31^{cd}	13.14	± 0.35^{bc}	22.85	± 0.39
Pak choi × Coconut fiber	2.08	± 0.13^{bc}	202.19	± 5.12^{bc}	9.76	± 0.43^{f}	23.97	± 0.49
Pak choi × Peat moss	3.16	± 0.14^{a}	200.02	± 16.64^{bc}	6.31	± 0.32^{g}	23.27	± 0.11
Significance								
Microgreens species (M)	***		ns		***		***	
Substrate (S)	***		***		***		**	
M × S	***		***		***		ns	

ns, **, *** Nonsignificant or significant at $p \leq 0.01$, and 0.001, respectively. Different superscript letters (a–g) within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

Coconut fiber was the second-best substrate in terms of yield for kohlrabi and pak choi but not for coriander, reflecting the sensitivity of coriander to the high initial EC levels encountered in this medium that resulted in delayed germination and slower growth rate (**Table 1; Figure 1**; Sánchez-Monedero et al., 2004; Okkaoğlu et al., 2015). Dry yield was not differentiated among species; however, it was significantly affected by the choice of substrate (**Table 2**). It was highest on peat moss, which exceeded that of the other four substrates by 35.7%. However, aside from peat moss, which maximized the yield of all species, ranking the rest four substrates for dry yield was confounded by M × S interaction. In terms of dry matter content, coriander microgreens attained the highest mean value (13.6%), which was 23.4% higher than the mean value of kohlrabi and pak choi. Microgreens grown on peat moss and coconut fiber had the lowest dry matter content overall, which was 31.1% lower than the average of the other four substrates. Nevertheless, significant M × S interaction indicated that species response to substrate for dry matter content was not uniform. For instance, contrary to kohlrabi and pak choi, the dry matter content of coriander microgreens was highest when grown on coconut fiber, cellulose sponge and capillary mat (**Table 2**), all of which were noted for their high EC content (**Table 1**). Increase in dry matter content in response to salt stress has been previously reported for other salt-sensitive species such as lettuce (Sánchez-Monedero et al., 2004; Carillo et al., 2019). Finally, canopy coloration, evaluated in terms of lightness (CIELAB parameter L*), incurred significant species and substrate effects without interaction. Microgreens canopy was progressively darker in the order coriander, kohlrabi, pak choi, expressed by significantly different and progressively lower L* values (**Table 2**). With respect to substrate, canopy color was significantly darker in microgreens grown on the synthetic substrates capillary mat and cellulose sponge compared to the natural fiber substrates of agave fiber, peat moss and coconut fiber. The lighter canopy color of microgreens grown on natural fibers is most probably linked to the faster growth and the generally lower dry matter content of microgreens grown on these media, which in turn is linked to their optimal aeration–water capacity balance (Savvas, 2007; Schmilewski, 2008; Di Gioia et al., 2017). Notwithstanding statistical differences, variation in canopy coloration was visually not readily perceptible, therefore it did not impact the marketable quality of microgreens.

3.3. Nitrate Concentration and Mineral Composition

Substrate effect on nitrate and mineral (P, K, Ca, Mg, S and Na) concentrations of microgreens differed across species as denoted by significant M × S interaction (**Table 3**). Overall, nitrate concentration was higher in the brassicaceous species pak choi (601.6 mg kg^{-1} fw) and kohlrabi (500.1 mg kg^{-1} fw) compared to coriander (398.5 mg kg^{-1} fw), which confirms that the general tendency of the *Brassicaceae* members for nitrate hyper-accumulation is as pertinent for microgreens as it is for their mature counterparts (Santamaria, 2006; Colla et al., 2018). Significant differences in microgreens' nitrate concentrations were also observed in response to substrate. In all species, the highest concentration was obtained on peat moss ($\bar{x}$ = 888.0 mg kg^{-1} fw), which was 54.6% higher than the mean concentration encountered in the rest substrates (403.1 mg kg^{-1} fw). Overall, the natural fiber substrates resulted in higher nitrate concentrations in microgreens compared to the synthetic substrates. Nitrate uptake is largely facilitated through the transpiration stream (Colla et al., 2018), therefore natural fibers abundant in macropores are expected to exert lower suction on water, which is readily available to facilitate higher transpiration and faster rates coupled with increased nitrate uptake (Raviv et al., 2002). The nitrate concentrations presently determined were substantially below the tolerance levels set for key salad crops (lettuce, spinach and rocket) by effective regulations (Commission of the European Communities, 2006); moreover, the limited quantity of microgreens consumed ensures that the daily toxic threshold (3.7 mg kg^{-1} body weight) set by the World Health Organization and the European Union is not easily violated (EFSA, 2008). Nitrate intake from various dietary sources is nonetheless cumulative and the current study indicates that nitrate deprivation practices applied on microgreens grown on natural fiber substrates, peat moss in particular, should be examined in order to minimize consumer exposure to nitrate residues. Among others, such practices may include reducing the nitrate concentration of the nutrient solution and reverting to a nitrate-free solution for 2–5 days before harvest (Colla et al., 2018; Kyriacou and Rouphael, 2018).

The role of macro-minerals in ameliorating nutritional deficiencies and maintaining homeostasis and metabolic functions in the human body has been well described (Gharibzahedi and Jafari, 2017). The dietary contribution of the macro-minerals K, Mg, Na, P, and Ca from vegetal sources has been estimated at approximately 35%, 24%, 11%, 11%

and 7%, respectively (Levander, 1990). The relative abundance of macrominerals in the microgreen species presently examined was largely in accordance with previous reports covering microgreens of wide-ranging botanical taxa (Xiao et al., 2016; Di Gioia et al., 2017a; Kyriacou et al., 2019a, 2019b), which in order of decreasing concentrations was K > Ca > P>Mg > S > Na. This finding contributes towards establishing a reference base for the nutritional value of microgreens in terms of mineral content, for which there remains a paucity of information. Moreover, little is known on the impact that different types of substrate may have on microgreens' mineral content. In the current study, natural fiber substrates including peat moss delivered microgreens of higher P concentration (4.26 ± 0.17 g kg^{-1} dw) than the synthetic substrates (3.83 ± 0.18 g kg^{-1} dw), notwithstanding significant M × S interaction (**Table 3**). Higher K content was obtained from microgreens grown on peat moss and coconut fiber; however, the synthetic capillary mat was also noted for delivering high K kohlrabi and pak choi microgreens. The highest Ca concentration in all species was clearly obtained in microgreens grown on peat moss, which was 54.2% higher than the mean concentration of the rest substrates. Peat moss also delivered coriander and kohlrabi microgreens of the highest Mg concentration, but substrate differences were more limited in the case of pak choi. Peat moss further sustained the highest S concentration in microgreens of all species. Finally, Na concentrations tended to be highest in microgreens grown on capillary mat and peat moss. In the former case, this stemmed from the high initial Na concentration of the capillary mat, whereas high evapotranspiration drove a buildup of Na in peat moss. Pronounced species differences in S concentration were observed, which was lower in coriander than kohlrabi and pak choi. High S content in the latter two species is unsurprising, since sulphur-rich glucosinolates constitute a signature trait of the *Brassicaceae* family (Neugart et al., 2018). Mg and P concentrations were the least variable across species. Of the three microgreens species examined, coriander was the species that accumulated the lowest concentrations of bothnitrate and minerals overall.

Table 3. Nitrate and mineral content of coriander, kohlrabi and pak choi substrates grown on natural fiber and synthetic substrates under a controlled environment. All data are expressed as mean ± standard error, $n = 3$.

Source of Variance	Nitrate	P	K	Ca	Mg	S	Na
Microgreens species (M)							
Coriander	398.5 ± 80.0^{c}	4.14 ± 0.25^{b}	15.60 ± 0.77^{b}	6.46 ± 1.26^{c}	4.27 ± 0.21	0.98 ± 0.17^{c}	0.48 ± 0.10^{c}
Kohlrabi	500.1 ± 59.6^{b}	3.93 ± 0.18^{b}	10.88 ± 0.69^{c}	12.98 ± 1.21^{a}	4.25 ± 0.21	3.10 ± 0.22^{b}	1.78 ± 0.37^{b}
Pak choi	601.6 ± 52.9^{a}	4.92 ± 0.29^{a}	21.20 ± 1.31^{a}	9.58 ± 0.73^{b}	4.22 ± 0.11	4.03 ± 0.52^{a}	2.68 ± 0.47^{a}
Substrate (S)							
Agave fiber	361.3 ± 48.5^{c}	4.68 ± 0.32^{a}	13.69 ± 1.52^{c}	8.04 ± 1.15^{c}	4.51 ± 0.19^{b}	2.23 ± 0.37^{bc}	0.44 ± 0.09^{d}
Capillary mat	324.3 ± 52.7$_{c}$	3.95 ± 0.38^{bc}	16.32 ± 1.94^{b}	6.12 ± 0.71^{d}	3.45 ± 0.16^{d}	2.64 ± 0.52^{b}	3.16 ± 0.66^{a}
Cellulose sponge	396.1 ± 60.7^{c}	3.70 ± 0.27^{c}	11.97 ± 1.29^{c}	9.43 ± 1.29^{b}	3.93 ± 0.14^{c}	1.82 ± 0.36$_{c}$	0.64 ± 0.14^{d}
Coconut fiber	530.5 ± 71.3^{b}	4.82 ± 0.20^{a}	19.06 ± 2.21^{a}	7.70 ± 0.89^{c}	4.42 ± 0.08^{b}	2.07 ± 0.38^{c}	1.25 ± 0.28^{c}
Peat moss	888.0 ± 31.8^{a}	4.49 ± 0.41^{ab}	18.43 ± 1.41^{a}	17.07 ± 1.16^{a}	4.92 ± 0.22^{a}	4.77 ± 0.80^{a}	2.75 ± 0.53^{b}
M × S							
Coriander × Agave fiber	236.8 ± 25.2^{ef}	5.17 ± 0.92^{ab}	15.62 ± 1.50^{efg}	3.94 ± 0.29^{h}	4.26 ± 0.49^{cde}	0.80 ± 0.10^{d}	0.17 ± 0.01^{g}
Coriander × Capillary mat	186.5 ± 43.2^{f}	3.53 ± 0.22^{cd}	13.82 ± 1.07^{efg}	3.58 ± 0.42^{h}	3.61 ± 0.12^{f}	0.81 ± 0.09^{d}	0.69 ± 0.03^{def}
Coriander × Cellulose sponge	255.4 ± 15.9^{ef}	3.20 ± 0.06^{cd}	12.18 ± 0.19^{gh}	4.39 ± 0.10^{h}	3.70 ± 0.09^{ef}	0.48 ± 0.05^{d}	0.14 ± 0.00^{g}
Coriander × Coconut fiber	333.1 ± 49.0^{def}	4.65 ± 0.24^{abc}	16.77 ± 1.03^{def}	4.58 ± 0.31$_{h}$	4.27 ± 0.17^{cde}	0.62 ± 0.04^{d}	0.33 ± 0.02^{fg}
Coriander × Peat moss	980.6 ± 37.2^{a}	4.15 ± 0.11^{bcd}	19.61 ± 0.60^{cd}	15.80 ± 0.68^{b}	5.53 ± 0.19^{a}	2.19 ± 0.09^{c}	1.09 ± 0.04^{de}
Kohlrabi × Agave fiber	301.3 ± 6.3^{ef}	4.53 ± 0.20^{abc}	8.03 ± 0.26i	11.79 ± 0.25^{de}	4.87 ± 0.16^{abc}	2.71 ± 0.08^{c}	0.36 ± 0.02efg
Kohlrabi × Capillary mat	276.4 ± 4.6^{ef}	3.06 ± 0.13^{d}	11.54 ± 0.48^{hi}	8.31 ± 0.30^{f}	2.89 ± 0.08^{g}	2.79 ± 0.11^{c}	4.01 ± 0.34^{b}
Kohlrabi × Cellulose sponge	399.8 ± 15.6^{cde}	3.95 ± 0.30^{bcd}	7.99 ± 0.36^{i}	12.84 ± 0.21^{cd}	3.90 ± 0.05^{def}	2.67 ± 0.19^{c}	0.71 ± 0.10^{defg}
Kohlrabi × Coconut fiber	729.0 ± 64.9^{b}	4.64 ± 0.10^{abc}	13.07 ± 0.83^{fgh}	10.60 ± 0.23^{e}	4.49 ± 0.11^{bcd}	2.63 ± 0.20^{c}	1.21 ± 0.14^{d}
Kohlrabi × Peat moss	793.8 ± 24.8^{ab}	3.46 ± 0.12^{cd}	13.76 ± 0.56^{efg}	21.35 ± 1.12^{a}	5.08 ± 0.07^{ab}	4.71 ± 0.09^{b}	2.59 ± 0.06^{c}
Pak choi × Agave fiber	545.8 ± 31.6^{c}	4.32 ± 0.36^{bcd}	17.43 ± 0.64^{de}	8.37 ± 0.45^{f}	4.39 ± 0.21^{cde}	3.17 ± 0.26^{c}	0.78 ± 0.05^{defg}
Pak choi × Capillary mat	509.9 ± 59.6^{cd}	5.28 ± 0.52^{ab}	23.59 ± 1.70^{b}	6.49 ± 0.11^{g}	3.83 ± 0.22^{def}	4.31 ± 0.34^{b}	4.79 ± 0.55^{a}
Pak choi × Cellulose sponge	533.0 ± 156.4^{c}	3.96 ± 0.77^{bcd}	15.74 ± 2.19^{efg}	11.06 ± 0.15^{e}	4.19 ± 0.39^{cde}	2.32 ± 0.36^{c}	1.07 ± 0.08^{def}
Pak choi × Coconut fiber	529.5 ± 123.2$_{c}$	5.19 ± 0.54^{ab}	27.33 ± 1.40^{a}	7.92 ± 0.56^{f}	4.52 ± 0.14^{bcd}	2.95 ± 0.24^{c}	2.22 ± 0.21^{c}
Pak choi × Peat moss	889.6 ± 37.4^{ab}	5.85 ± 0.69^{a}	21.91 ± 2.39^{bc}	14.08 ± 0.15^{c}	4.16 ± 0.24^{cde}	7.41 ± 0.93^{a}	4.56 ± 0.54^{ab}
Significance							
Microgreens species (M)	***	***	***	***	ns	***	***
Substrate (S)	***	**	***	***	***	***	***
M × S	***	**	**	***	***	***	***

ns,**, *** Nonsignificant or significant at $p \leq 0.01$, and 0.001, respectively. Different superscript letters (a–i) within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.4. Chlorophyll, Carotenoid and Ascorbate Content

The total chlorophyll content of microgreens varied more with respect to species (8.85–14.33 mg kg^{-1} fw) than substrate (11.16–13.13 mg kg^{-1} fw; **Table 4**). These levels were nearly two-fold higher than those found by Samuolienė et al. (2017) in kohlrabi, mibuna and mustard microgreens. Other than varietal, this difference possibly reflects harvest performed at different developmental stages, i.e., at the cotyledonary stage, at the appearance of the first or second true leaf, the latter being the canonical stage for microgreens (Kyriacou et al., 2016a). Kohlrabi had significantly lower total chlorophyll content that amounted to only 63.3% of the mean content found in coriander and pak choi microgreens. Higher chlorophyll content was obtained from microgreens grown on synthetic capillary mat and cellulose sponge than peat moss and coconut fiber, whereas intermediate levels were obtained on agave fiber. These substrate effects were similar to those manifested on canopy color and are likely associated with the higher growth rate and lower dry matter content of microgreens grown on natural fiber substrates which sustain optimal moisture–aeration conditions (Savvas, 2007; Schmilewski, 2008; Di Gioia et al., 2017).

The content of microgreens in the major carotenoid compounds lutein and β-carotene contributes to their bioactive value, as both molecules are lipophilic antioxidants drawing light-absorbing and ROS-quenching properties from their long polyene chains (Young and Lowe, 2001). Dietary supplementation with lutein has been associated with macular protection against oxidative damage and degeneration (Kvansakul et al., 2006); whereas β-carotene is a precursor of vitamin A, essential for growth, visual and immune functions. In the microgreen species presently examined, lutein content was highest in pak choi and lowest in kohlrabi (122.6 and 73.2 mg kg^{-1} fw, respectively; **Table 4**). Wide variation in lutein content was also observed between microgreens grown on cellulose sponge (70.4 mg kg^{-1} fw) and those grown on the rest four media ($\bar{x}$ =105.1 mg kg^{-1} fw). Beta-carotene content was highest in coriander, followed by pak choi and then kohlrabi. Significant M × S interaction confounded the substrate effect. In the case of coriander, β-carotene was highest in microgreens grown on capillary mat; whereas, in kohlrabi and pak choi microgreens, differences between substrates were not statistically significant. Variability in the levels of lutein and β-carotene was higher in relation to species than in response to substrate.

Microgreens' ascorbate content was overall highest in kohlrabi, followed by pak choi and lowest in coriander (**Table 4**). However, significant M × S interaction was observed as species responded differently to the five substrates examined. Ascorbate content in coriander microgreens was significantly lower on peat moss compared to the other media. In kohlrabi microgreens, ascorbate content was lower on peat moss and coconut fiber compared to agave fiber, capillary mat and cellulose sponge. Finally, cellulose sponge was the substrate that delivered the highest ascorbate content in pak choi microgreens, whereas the other four substrates had non-significant differences among them.

Table 4. Chlorophyll, lutein, *β*-carotene and ascorbate content of coriander, kohlrabi and pak choi substrates grown on natural fiber and synthetic substrates under a controlled environment. All data are expressed as mean ± standard error, $n = 3$.

Source of Variance	Total Chlorophyll (mg kg^{-1} fw)	Lutein (mg kg^{-1} dw)	*β*-Carotene (mg kg^{-1} dw)	Total Ascorbic Acid
Microgreens species (M)				
Coriander	13.63 ± 0.38^{a}	98.6 ± 8.8^{b}	325.1 ± 38.1^{a}	121.40 ± 5.77^{c}
Kohlrabi	8.85 ± 0.37^{b}	73.2 ± 5.9^{c}	183.1 ± 15.5^{c}	199.87 ± 8.77^{a}
Pak choi	14.33 ± 0.59^{a}	122.6 ± 7.5^{a}	236.8 ± 19.0^{b}	177.12 ± 5.38^{b}
Substrate (S)				
Agave fiber	12.59 ± 0.90^{ab}	109.5 ± 15.7^{a}	233.2 ± 31.6^{b}	179.79 ± 12.16^{a}
Capillary mat	13.13 ± 0.89^{a}	112.3 ± 9.8^{a}	351.1 ± 56.0^{a}	173.37 ± 13.46^{a}
Cellulose sponge	13.07 ± 1.17^{a}	70.4 ± 8.2^{b}	153.8 ± 11.0^{c}	187.39 ± 16.08^{a}
Coconut fiber	11.16 ± 0.89^{b}	103.3 ± 8.6^{a}	250.3 ± 28.9^{b}	150.35 ± 9.77^{b}
Peat moss	11.39 ± 1.17^{b}	95.1 ± 11.0^{a}	253.2 ± 23.9^{b}	139.75 ± 14.03^{b}
M × S				
Coriander × Agave fiber	12.70 ± 1.04	121.0 ± 23.5	312.9 ± 36.0^{bc}	146.16 ± 3.23^{bcd}
Coriander × Capillary mat	15.03 ± 0.84	99.4 ± 15.8	533.3 ± 103.1^{a}	128.92 ± 4.93^{cd}
Coriander ×Cellulose sponge	13.76 ± 0.24	57.8 ± 7.2	169.1 ± 18.6^{def}	128.01 ± 6.92^{cd}
Coriander × Coconut fiber	13.78 ± 0.18	120.4 ± 13.2	355.2 ± 25.4^{b}	116.41 ± 8.80^{d}
Coriander × Peat moss	12.89 ± 1.22	94.3 ± 19.3	255.0 ± 33.3^{bcd}	87.52 ± 7.88^{e}
Kohlrabi × Agave fiber	9.68 ± 0.32	58.0 ± 7.3	134.9 ± 16.7^{ef}	219.14 ± 19.37^{a}
Kohlrabi × Capillary mat	10.03 ± 0.99	101.9 ± 16.5	234.5 ± 29.3^{bcd}	217.77 ± 13.18^{a}
Kohlrabi × Cellulose sponge	9.35 ± 0.30	52.2 ± 4.2	122.6 ± 15.9^{f}	226.75 ± 11.88^{a}
Kohlrabi × Coconut fiber	7.85 ± 0.23	73.8 ± 6.5	180.8 ± 18.6^{cde}	158.42 ± 11.07^{bc}
Kohlrabi × Peat moss	7.34 ± 0.92	79.8 ± 8.2	242.7 ± 22.9^{bcd}	177.27 ± 7.44b
Pak choi × Agave fiber	15.40 ± 0.50	149.4 ± 13.3	252.0 ± 46.8^{bcd}	174.08 ± 5.81^{b}
Pak choi × Capillary mat	14.34 ± 0.77	135.5 ± 14.8	285.6 ± 24.9^{bcd}	173.42 ± 1.85^{b}
Pak choi × Cellulose sponge	16.09 ± 2.10	101.2 ± 3.6	169.6 ± 10.9^{def}	207.41 ± 13.28^{a}
Pak choi × Coconut fiber	11.86 ± 0.50	115.7 ± 4.7	214.9 ± 22.1^{cde}	176.22 ± 1.28^{b}
Pak choi × Peat moss	13.94 ± 1.19	111.2 ± 27.8	261.9 ± 71.7^{bcd}	154.45 ± 8.26^{bc}
Significance				
Microgreens species (M)	***	***	***	***
Substrate (S)	*	**	***	***
M × S	ns	ns	*	*

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different superscript letters (a–f) within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

3.5. Phenolic Composition

Chromatographic separation and quantitation of phenolic compounds by Q Exactive Orbitrap LC-MS/MS enabled the assessment of twenty constituent polyphenolic profiles for the three species of microgreens evaluated (**Table 5**). The total sum of polyphenols was highest in kohlrabi (16.26 ± 0.78 mg g^{-1} dw) and lowest in pak choi (14.22 ± 0.78 mg g^{-1} dw), and it was overall higher than the range reported by Xiao et al. (2015) (1.5–7.0 mg g^{-1} dw) and Bulgari et al. (2017) (164–328 µg g^{-1} fw) through spectroscopic assessment of various species of microgreens. This might reflect variable developmental stages at harvest, genotypic differences and also differences in analytical methodology. Moreover, variation in the method of harvest applied and the severity of the mechanical trauma inflicted can affect phenolic accumulation via induced signaling that migrates to adjacent nonwounded tissue wherein it triggers respiratory climax and phenylpropanoid biosynthesis (Saltveit et al., 2005). Comparatively wider variability in total polyphenols was encountered across substrate types than species of microgreens; significant interaction however highlighted a species-dependent response to substrate type (**Table 5**).

Table 5. Phenolic composition of coriander, kohlrabi and pak choi substrates grown on natural fiber and synthetic substrates under a controlled environment. All data are expressed as mean ± standard error, $n = 3$.

Source of variance	Caffeic acid (µg g^{-1} dw)	Caffeic acid hexoside (µg g^{-1} dw)	Chlorogenic acid (µg g^{-1} dw)	*p*-Coumaroylquinic acid (µg g^{-1} dw)	Dicaffeoylquinic acid (µg g^{-1} dw)	Ferulic acid (µg g^{-1} dw)	Feruloyl quinic acid (µg g^{-1} dw)
Microgreens species (M)							
Coriander	125.84 ± 16.7 a	88.53 ± 4.1 a	6367 ± 549 b	64.85 ± 2.11 a	33.52 ± 1.22 a	20.55 ± 0.04 a	533.8 ± 20.6 a
Kohlrabi	40.34 ± 4.0 b	38.24 ± 4.5 b	6776 ± 516 b	22.99 ± 0.20 c	19.72 ± 0.03 b	19.55 ± 0.01 c	78.95 ± 3.3 c
Pak choi	32.71 ± 1.1 c	38.69 ± 3.1 b	9026 ± 837 a	25.90 ± 0.62 b	19.59 ± 0.03 b	20.24 ± 0.03 b	117.66 ± 8.4 b
Substrate (S)							
Agave fiber	48.79 ± 7.2 d	55.07 ± 8.1 b	9947 ± 1095 a	40.40 ± 4.78 ab	23.75 ± 1.39 b	20.12 ± 0.11 b	281.26 ± 54.2 a
Capillary mat	56.56 ± 4.7 c	49.05 ± 5.9 bc	8625 ± 780 b	42.01 ± 5.66 a	22.98 ± 1.14 b	20.08 ± 0.10 b	260.1 ± 48.2 ab
Cellulose sponge	119.46 ± 31.6 a	49.87 ± 6.9 bc	8757 ± 579 b	32.47 ± 3.17 d	24.74 ± 1.70 b	20.05 ± 0.09 b	210.8 ± 42.2 c
Coconut fiber	40.81 ± 7.1 e	43.28 ± 9.6 c	5290 ± 466 c	37.85 ± 5.06 bc	26.98 ± 2.94 a	20.22 ± 0.13 a	229.8 ± 60.8 bc
Peat moss	65.86 ± 4.4 b	78.49 ± 3.2 a	4331 ± 500 d	36.84 ± 5.21 c	22.95 ± 1.18 b	20.11 ± 0.11 b	235.5 ± 54.7 bc
M × S							
Coriander × Agave fiber	86.11 ± 9.7 b	100.90 ± 5.7 a	8244 ± 1149 de	67.41 ± 2.82 b	31.82 ± 0.32 bc	20.53 ± 0.08 bc	587.9 ± 25.4 a
Coriander × Capillary mat	78.25 ± 0.5 bc	82.48 ± 2.6 a	4807 ± 762 gh	73.92 ± 4.53 a	29.45 ± 0.88 c	20.38 ± 0.10 cd	537.0 ± 19.9 a
Coriander x Cellulose sponge	303.39 ± 3.0 a	83.80 ± 9.9 a	9850 ± 679 cd	49.45 ± 3.88 c	34.60 ± 0.61 b	20.46 ± 0.04 cd	434.1 ± 53.1 b
Coriander × Coconut fiber	76.75 ± 10.6 bc	89.20 ± 17.0 a	4113 ± 728 h	66.64 ± 3.34 b	41.98 ± 4.55 a	20.73 ± 0.10 a	560.7 ± 69.8 a
Coriander × Peat moss	84.70 ± 1.4 b	86.24 ± 3.3 a	4822 ± 852 gh	66.81 ± 2.72 b	29.78 ± 0.47 c	20.66 ± 0.09 ab	549.5 ± 28.6 a
Kohlrabi x Agave fiber	23.73 ± 0.3 gh	28.60 ± 0.5 cd	5846 ± 164 gh	22.95 ± 0.11 e	19.72 ± 0.02 d	19.49 ± 0.00 f	76.37 ± 2.8 de
Kohlrabi × Capillary mat	59.64 ± 1.9 d	36.52 ± 1.0 c	11974 ± 601 b	24.77 ± 0.21 de	19.95 ± 0.02 d	19.54 ± 0.01 f	97.49 ± 4.3 de
Kohlrabi × Cellulose sponge	30.18 ± 0.9 efg	29.33 ± 0.6 cd	5724 ± 200 gh	22.50 ± 0.16 e	19.81 ± 0.02 d	19.55 ± 0.01 f	68.37 ± 3.2 de
Kohlrabi × Coconut fiber	17.01 ± 0.5 h	12.96 ± 0.4 d	4176 ± 223 h	21.86 ± 0.27 e	19.49 ± 0.00 d	19.50 ± 0.00 f	55.75 ± 2.1 e
Kohlrabi × Peat moss	71.12 ± 1.4 c	83.79 ± 1.4 a	6162 ± 134 fg	22.87 ± 0.13 e	19.65 ± 0.01 d	19.64 ± 0.01 f	96.79 ± 2.9 de
Pak choi × Agave fiber	36.53 ± 0.7 ef	35.70 ± 0.7 c	15750 ± 462 a	30.84 ± 0.22 d	19.71 ± 0.01 d	20.33 ± 0.03 d	179.5 ± 2.2 c
Pak choi × Capillary mat	31.80 ± 0.4 efg	28.16 ± 0.6 cd	9093 ± 256 cde	27.33 ± 0.38 de	19.54 ± 0.00 d	20.30 ± 0.06 d	145.9 ± 5.1 cd
Pak choi × Cellulose sponge	24.80 ± 0.2 fgh	36.49 ± 6.6 c	10697 ± 296 bc	25.45 ± 0.50 de	19.81 ± 0.01 d	20.13 ± 0.01 e	129.8 ± 3.2 cde
Pak choi ×x Coconut fiber	28.66 ± 0.6 fgh	27.68 ± 0.6 cd	7580 ± 253 ef	25.05 ± 0.46 de	19.49 ± 0.00 d	20.42 ± 0.02 cd	72.90 ± 1.7 de
Pak choi × Peat moss	41.77 ± 0.4 e	65.44 ± 6.4 b	2009 ± 87 i	20.83 ± 0.10 e	19.41 ± 0.00 d	20.03 ± 0.02 e	60.23 ± 2.0 e
Significance							
Microgreens species (M)	***	***	***	***	***	***	***
Substrate (S)	***	***	***	***	***	**	**
M × S	***	***	***	***	***	***	**

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different superscript letters (a–h) within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

Table 5. *Cont.*

Source of variance	Feruloylglycoside (µg g^{-1} dw)	Kaempferol-3-O-(caffeoyl) sophoroside-7-O-glucoside (µg g^{-1} dw)	Kaempferol-3-O-(feruloyl) sophoroside-7-O-glucoside (µg g^{-1} dw)	Kaempferol-3-O-(synapil) sophoroside-7-O-glucoside (µg g^{-1} dw)	Kaempferol-3-O-rutinoside (µg g^{-1} dw)	Luteolina-3-O-rutinoside (µg g^{-1} dw)	Quercetin sophoroside (µg g^{-1} dw)
Microgreens species (M)							
Coriander	93.14 ± 2.6 c	nd	319.0 ± 44.7 a	86.9 ± 14 c	566.5 ± 51 a	563 ± 51.3 a	83.99 ± 6.0 a
Kohlrabi	154.79 ± 17.0 b	25.47 ± 1.44 b	169.9 ± 6.3 b	291.7 ± 10 b	114.1 ± 3 b	164 ± 21.0 b	4.40 ± 0.1 c
Pak choi	314.81 ± 50.7 a	35.56 ± 2.25 a	113.4 ± 8.4 c	862.5 ± 40 a	128.4 ± 5 b	146 ± 8.3 b	10.80 ± 0.3 b
Substrate (S)							
Agave fiber	84.61 ± 4.6 d	16.68 ± 0.89 c	136.4 ± 5.3 c	363.4 ± 70 c	265.3 ± 43 a	238 ± 47.6 c	43.05 ± 12.6 a
Capillary mat	107.30 ± 9.6 c	32.90 ± 1.03 b	180.8 ± 18.1 b	512.5 ± 125 a	321.6 ± 80 a	340 ± 76.5 ab	26.88 ± 6.8 b
Cellulose sponge	272.90 ± 53.0 b	34.61 ± 1.06 ab	143.1 ± 13.6 c	446.2 ± 85 b	156.9 ± 20 b	223 ± 12.9 c	44.30 ± 13.1 a
Coconut fiber	94.75 ± 3.4 d	30.94 ± 3.59 b	363.1 ± 71.1 a	362.0 ± 63 c	304.8 ± 73 a	260 ± 79.0 bc	28.56 ± 7.3 b
Peat moss	378.32 ± 60.8 a	37.45 ± 4.35 a	180.3 ± 23.7 b	384.5 ± 66 c	299.7 ± 74 a	394 ± 63.3 a	22.53 ± 5.7 b
M × S							
Coriander × Agave fiber	86.11 ± 2.4 efg	nd	116.2 ± 2.7 f	99.8 ± 15 g	505.2 ± 40 b	502 ± 39.8 bc	114.72 ± 7.9 a
Coriander × Capillary mat	84.89 ± 5.2 fg	nd	283.6 ± 1.9 b	55.0 ± 14 g	741.7 ± 108 a	738 ± 108.4 a	64.98 ± 5.4 bc
Coriander x Cellulose sponge	82.59 ± 1.6 fg	nd	137.8 ± 2.7 ef	94.5 ± 26 g	250.4 ± 37 c	243 ± 37.3 de	117.17 ± 12.3 a
Coriander × Coconut fiber	102.25 ± 3.5 ef	nd	769.1 ± 44.9 a	50.4 ± 16 g	644.4 ± 139 ab	641 ± 139.2 ab	69.25 ± 6.9 b
Coriander × Peat moss	109.85 ± 5.6 e	nd	288.1 ± 5.8 b	134.8 ± 54 g	690.8 ± 103 a	689 ± 102.8 a	53.83 ± 6.2 c
Kohlrabi x Agave fiber	62.60 ± 1.1 g	15.96 ± 1.75 e	130.8 ± 4.1 ef	228.3 ± 11 f	127.8 ± 3 c	84.6 ± 18.5 ef	4.22 ± 0.1 d
Kohlrabi × Capillary mat	159.55 ± 9.4 d	33.24 ± 0.80 c	138.8 ± 3.9 ef	258.4 ± 4 ef	100.2 ± 3 c	154 ± 3.7 ef	4.41 ± 0.1 d
Kohlrabi × Cellulose sponge	158.42 ± 3.7 d	33.94 ± 0.74 c	213.5 ± 3.5 c	343.2 ± 18 de	109.8 ± 5 c	207 ± 10.0 def	4.28 ± 0.1 d
Kohlrabi × Coconut fiber	78.18 ± 2.8 g	19.56 ± 1.25 e	166.8 ± 2.9 de	357.3 ± 12 d	128.4 ± 4 c	28.1 ± 0.7 f	4.58 ± 0.1 d
Kohlrabi × Peat moss	315.19 ± 15.9 c	24.66 ± 1.74 d	199.7 ± 7.4 cd	271.3 ± 5 def	104.3 ± 2 c	350 ± 10.6 cd	4.52 ± 0.0 d
Pak choi × Agave fiber	105.13 ± 5.0 ef	17.40 ± 0.50 e	162.3 ± 5.9 de	762.1 ± 31c	163.0 ± 2 c	129 ± 11.2 ef	10.22 ± 0.1 d
Pak choi × Capillary mat	77.46 ± 1.6 g	32.56 ± 1.99 c	120.0 ± 11.6 f	1224.0 ± 44 a	122.8 ± 7 c	129 ± 18.3 ef	11.23 ± 0.4 d
Pak choi × Cellulose sponge	577.69 ± 14.7 b	35.29 ± 2.05 c	78.0 ± 5.0 g	900.9 ± 65 b	110.7 ± 1 c	219 ± 7.6 de	11.46 ± 0.9 d
Pak choi ×x Coconut fiber	103.81 ± 3.7 ef	42.32 ± 1.82 b	153.5 ± 6.7 ef	678.4 ± 16 c	141.5 ± 8 c	112 ± 4.0 ef	11.85 ± 0.6 d
Pak choi × Peat moss	709.93 ± 13.4 a	50.24 ± 3.84 a	53.0 ± 3.0 g	747.3 ± 20 c	103.9 ± 1 c	144 ± 2.6 ef	9.25 ± 0.6 d
Significance							
Microgreens species (M)	***	***	***	***	***	***	***
Substrate (S)	***	***	***	***	**	***	***
M × S	***	***	***	***	***	***	***

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different superscript letters (a–h) within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

Table 5. *Cont.*

Source of variance	Quercetin-3-O-(feruloyl) sophoroside-7-O-glucoside (µg g^{-1} dw)	Quercetin-3-O-sophoroside-7-O-glucoside (µg g^{-1} dw)	Quercetina-3-O-glucoside (µg g^{-1} dw)	Quercetina-3-O-glucuronide (µg g^{-1} dw)	Rosmarinic acid (µg g^{-1} dw)	Rutin (µg g^{-1} dw)	Total polyphenols (mg g^{-1} dw)
Microgreens species (M)							
Coriander	nd	4.89 ± 0.08 c	381.4 ± 23.9 a	791.1 ± 48 a	20.41 ± 0.08 c	5109 ± 367 b	15.25 ± 0.57 ab
Kohlrabi	10.79 ± 0.40 b	30.45 ± 1.08 b	222.0 ± 8.3 b	nd	20.64 ± 0.03 b	8052 ± 735 a	16.26 ± 0.78 a
Pak choi	45.50 ± 2.93 a	71.93 ± 4.02 a	95.4 ± 6.0c	nd	20.78 ± 0.05 a	3096 ± 365 c	14.22 ± 0.78 b
Substrate (S)							
Agave fiber	18.55 ± 3.14 e	21.99 ± 3.37 c	216.3 ± 40.7 bc	754.1 ± 46 bc	20.52 ± 0.07 b	4027 ± 581 d	16.11 ± 0.71 a
Capillary mat	35.98 ± 6.7 b	33.97 ± 6.30 b	239.2 ± 33.1 ab	1057.9 ± 90 a	20.46 ± 0.07 b	5061 ± 390 bc	16.32 ± 0.39 a
Cellulose sponge	22.35 ± 3.53 d	35.45 ± 6.10 b	274.8 ± 35.4 a	580.6 ± 66 c	20.61 ± 0.05 b	4584 ± 586 cd	15.65 ± 0.44 a
Coconut fiber	39.51 ± 9.02 a	43.40 ± 9.53 a	171.1 ± 17.1 c	617.5 ± 98 c	20.95 ± 0.09 a	5646 ± 543 b	13.24 ± 0.75 b
Peat moss	24.34 ± 3.96 c	43.97 ± 8.71 a	263.4 ± 35.4 ab	945.6 ± 84 ab	20.51 ± 0.07 b	7776 ± 1377 a	14.91 ± 1.68 a
M × S							
Coriander × Agave fiber	nd	5.11 ± 0.17 h	430.1 ± 49.5 a	754.1 ± 46 b	20.18 ± 0.04 h	5169 ± 344 cde	16.94 ± 1.35 bcd
Coriander × Capillary mat	nd	4.80 ± 0.17 h	378.8 ± 42.1 a	1057.9 ± 90 a	20.18 ± 0.07 h	6741 ± 813 bc	15.80 ± 0.80 bcdef
Coriander x Cellulose sponge	nd	5.28 ± 0.14 h	428.9 ± 68.1 a	580.6 ± 66 c	20.49 ± 0.13 ef	2800 ± 474 fg	
Coriander × Coconut fiber	nd	4.66 ± 0.15 h	235.4 ± 18.0 b	617.5 ± 98 c	20.95 ± 0.24 ab	5427 ± 996 cde	13.55 ± 1.93 efg
Coriander × Peat moss	nd	4.57 ± 0.18 h	434.0 ± 40.3 a	945.6 ± 84 a	20.2 ± 0.11 gh	5406 ± 530 cde	14.44 ± 0.43 defg
Kohlrabi x Agave fiber	8.13 ± 0.19 h	22.14 ± 0.71 g	150.5 ± 3.8 cd	nd	20.5 ± 0.03 def	6158 ± 11 cd	13.05 ± 0.13 fg
Kohlrabi × Capillary mat	13.89 ± 0.69 f	30.48 ± 1.64 ef	262.4 ± 8.1 b	nd	20.4 ± 0.04 fgh	4181 ± 128 ef	17.59 ± 0.59 bc
Kohlrabi × Cellulose sponge	10.94 ± 0.50 g	34.65 ± 1.42 de	245.5 ± 7.0 b	nd	20.7 ± 0.04 bcdef	7852 ± 202 b	
Kohlrabi × Coconut fiber	9.65 ± 0.23 gh	28.82 ± 0.94 f	197.8 ± 4.9 bc	nd	20.7 ± 0.06 bcde	6601 ± 41 bcd	11.96 ± 0.26 g
Kohlrabi × Peat moss	11.34 ± 0.42 g	36.16 ± 1.80 d	253.8 ± 7.0 b	nd	20.8 ± 0.03 bc	15467 ± 660 a	23.53 ± 0.64 a
Pak choi × Agave fiber	28.96 ± 0.27 e	38.71 ± 1.45 d	68.2 ± 1.2 d	nd	20.8 ± 0.03 bc	753 ± 35 h	18.33 ± 0.49 b
Pak choi × Capillary mat	58.07 ± 1.53 b	66.64 ± 4.02 c	76.4 ± 1.9 d	nd	20.8 ± 0.03 bcd	4261 ± 157 ef	15.57 ± 0.25 cdef
Pak choi × Cellulose sponge	33.77 ± 1.61 d	66.42 ± 2.05 c	150.1 ± 6.1 cd	nd	20.7 ± 0.04 cdef	3100 ± 140 fg	16.26 ± 0.42 bcde
Pak choi ×x Coconut fiber	69.36 ± 1.26 a	96.72 ± 3.75 a	80.0 ± 2.6 d	nd	21.2 ± 0.05 a	4912 ± 1308 de	14.20 ± 1.19 defg
Pak choi × Peat moss	37.34 ± 1.11 c	91.17 ± 1.51 b	102.3 ± 10.3 d	nd	20.4 ± 0.04 efg	2454 ± 56 g	6.76 ± 0.13 h
Significance							
Microgreens species (M)	***	***	***	***	***	***	***
Substrate (S)	***	***	***	***	***	***	***
M × S	***	***	**	***	***	***	***

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different superscript letters (a–h) within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

Coriander and pak choi microgreens exhibited analogous responses as their phenolic content decreased on peat moss and coconut fiber. The same response was observed in Kohlrabi microgreens grown on coconut fiber, intriguingly though the highest phenolic content was obtained on peat moss. The response encountered in coriander and pak choi microgreens can be readily interpreted in the context of the faster growth rate and lack of physiological stress afforded by the optimal root environment in peat moss and coconut fiber substrates (**Table 1; Figure 1A-C**).

Of the twenty phenolic compounds quantitated, chlorogenic acid and rutin (quercetin-3-*O*-rutinoside) were the most abundant, accounting respectively for 48.5% and 35.5% of the total phenolic concentration (**Table 5**). The abundance of chlorogenic acid in young plant tissues and its decline at subsequent developmental stages has been demonstrated by Vallejo et al. (2003) on *Brassica* seedlings, and probably relates to the intermediary role of chlorogenic acid in lignin biosynthesis (Boerjan et al., 2003). Aside from this role, the abundance of chlorogenic acid in microgreens underpins their bioactive value based on chlorogenic acid's antihypertensive effects on arterial pressure (Onakpoya et al., 2015), and its putative anti-inflammatory action (Tajik et al., 2017). Previous work on eggplant has shown chlorogenic acid as the main contributor of in vitro antioxidant capacity, subject to genetic and environmental influence, such as developmental stage, genotype and cultural practices (Whitaker and Stommel, 2003). A recent study by Santos et al. (2020) demonstrated that the inhibition of lipoperoxidation, A549 cell proliferation and antihypertensive activity were highest in plant extracts having chlorogenic acid as a major phenolic constituent. Similarly, the analgesic and anti-inflammatory activities of ethyl acetate extracts of *Kleinia pendula* (Forssk.) DC. were attributed to their phenolic acid content that was particularly rich in chlorogenic acid (Alfaifi et al., 2020). Santos et al. (2020) also demonstrated that the ex vivo activity against low-density lipoprotein (LDL) oxidation, the protection of human erythrocytes and the cytotoxic-antiproliferative activity against HCT8 cancer cells was high in plant aqueous extracts rich in quercetin-3-rutinoside (rutin). In the present study, the mean phenolic content of microgreens across the species was made up of hydroxycinnamic acids and their derivatives by 49.8%, flavonol glycosides by 48.4% and flavone glycosides by 1.8%. This breakdown of polyphenolic constitution is relatively lower on hydroxycinnamic acids and higher on flavonol glycosides than

previously found in cress, amaranth and mizuna microgreens (Kyriacou et al., 2019a, 2019b), and reflects genotypic variation even within the *Brassicaceae* microgreens, as well as variation possibly introduced by slight differences in harvest maturity. It is in line, however, with the findings of previous researchers who demonstrated the predominance of quercetin and kampferol *O*-glycosides in the flavonol fraction of Brassica microgreens polyphenols (Terao et al., 2001; Cartea et al., 2011) In this respect, the absence of kaempferol-3-*O*-(caffeoyl) sophoroside-7-*O*-glucoside and quercetin-3-*O*-glucuronide from coriander microgreens is unsurprising. Aside from the overall phenolic content, the presence of quercetin-3-*O*-glucuronide in the two brassicaceous microgreens (pak choi and kohlrabi) is important in view of studies demonstrating its protective role on dietary antioxidants found in the human plasma (Olsen et al., 2009). A recent study by Lesjak et al. (2018) demonstrated that quercetin derivatives, such as quercetin-3-*O*-glucuronide, entering systemic circulation after their consumption may exert antioxidant and anti-inflammatory activity, thus highlighting the overall nutraceutical value of a quercetin-rich diet. Despite certain outstanding differences observed between species in the mean concentration of particular polyphenols (e.g., higher mean concentration of rutin in kohlrabi; of caffeic acid and kaempferol-3-*O*-rutinoside in coriander; of chlorogenic acid and kaempferol-3-*O*-(synapil)sophoroside-7-*O*-glucoside in pak choi), significant species–substrate interaction confounded putative species signature traits and complicated the interpretation of substrate effects with respect to particular phenolic constituents. Given the heightened respiratory and metabolic activity encountered in the rapidly growing and differentiating tissues of microgreens (Kyriacou et al., 2016a), even minimal differences in the stages of ontogeny at harvest may arrest disparate states of transient phenylpropanoid components thereby introducing qualitative variation in polyphenolic profiles, as previously demonstrated for mineral constituents during seedling ontogeny (Pinto et al., 2015). It is apparent nonetheless that substrates providing optimal environment for microgreens growth, such as peat moss, tend to enhance growth rate and biomass production at the expense of overall phenolic content. In this respect, the application of controlled stress (eustress) on microgreens growing on peat moss merits investigation as a means of enhancing phytochemical composition without significantly compromising crop performance and production turnover.

3.6. Principal Component Analysis of Nutritional and Functional Quality Parameters in Response to Substrate for Coriander, Kohlrabi and Pak Choi Microgreens

Principal component analysis (PCA) has been previously demonstrated as an effective way of collectively representing sample population differences over multiple traits of productivity and quality in response to numerous cultivation factors (El-Nakhel et al., 2019; Kyriacou et al., 2019a, 2019b). In the current study, the PCA enabled a summarized view of the relations between microgreens crop performance and the compositional variables assessed (**Figure 2A–C**). Owing to the significant species-substrate interaction observed on most of these variables, PCA analysis was performed separately for each species. As a result, the species-dependent effects of the five substrates were better visualized and the quality of the PCA loading and score plots were improved, as indicated by the high percentage of the total variance (76.7–85.7%) accounted for the first two PCs. The main conclusive evidence provided with respect to the three species examined is the superiority of peat moss over other substrates in terms of microgreen yield and overall mineral content; however, peat moss also resulted in higher nitrate and lower dry matter content in microgreens across species. Chlorophyll content was particularly enhanced in microgreens grown on capillary mat and cellulose sponge. On the contrary, the concentrations of total polyphenols and major carotenoid molecules varied across substrates in a species-specific manner. For instance, total polyphenols were lowest on peat moss for coriander and pak choi microgreens, whereas kohlrabi microgreens grown on peat moss had the highest polyphenols. Therefore, the PCA representations underpin the main conclusion derived from the tabulated results (**Tables 2–5**) that although the superiority of peat moss in several productive and compositional traits of microgreens is demonstrable, further research work is warranted to elucidate the interactive effects of substrate on certain quality traits of particular species. Although the synthetic substrates presently examined do represent competitive alternatives to peat moss, particularly in terms of growth rate, fresh yield and microgreens mineral composition, research work on finding viable alternatives to peat moss is warranted by sustainability issues associated with peat moss extraction (Barrett et al., 2016), and also due to microbial safety aspects associated with the use of organic materials such as peat moss for microgreens production (Di Gioia et al., 2017).

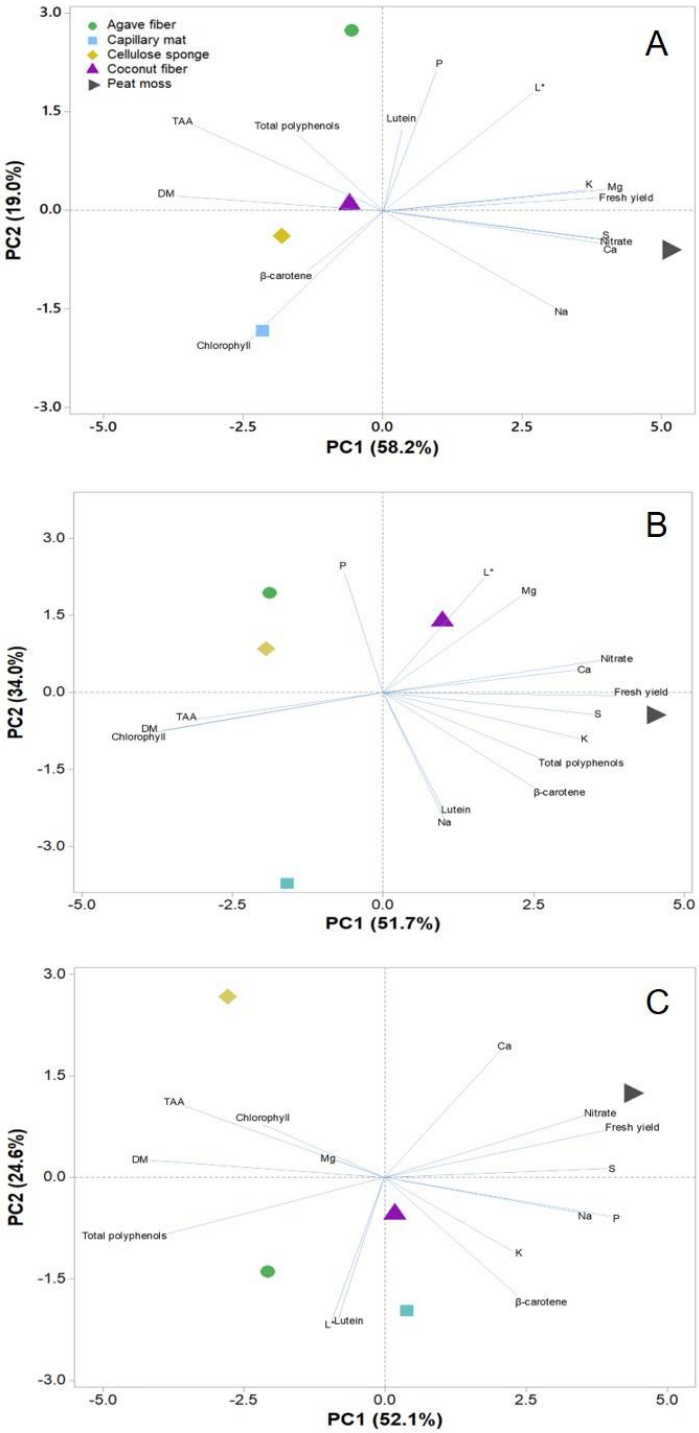

Figure 2. Principal component analysis loading plot of yield, mineral and phytochemical composition traits of coriander (A), kohlrabi (B) and pak choi (C) microgreens cultivated on natural fiber and synthetic substrates in a controlled growth environment.

Conclusions

The current work constitutes a novel and unprecedented report on how the physicochemical properties of natural fiber and synthetic fiber substrates can influence the phytochemical content of microgreens. A key finding of the present work, which advances our understanding of the current and future literature on microgreens production and potential bioactive value, is that substrates which combine optimal physicochemical properties, such as peat moss, tend to promote faster growth and higher fresh yields that favor high production turnover; however, this is achieved at the expense of reduced phytochemical content, the foremost of polyphenols. Therefore, controlled stress applications (e.g., osmotic stress) on microgreens growing on such media warrants investigation as a means of enhancing phytochemical composition without substantial compromise in crop performance and production turnover. Substrates promoting fast growth (e.g., peat moss) also tend to promote nitrate accumulation in microgreens, especially in brassicaceous ones that are known nitrate hyperaccumulators. Therefore, nitrate deprivation practices should be considered for microgreens grown on such substrates in order to minimize consumer exposure to nitrates.

Chapter 5: Ontogenetic Variation in the Mineral, Phytochemical and Yield Attributes of Brassicaceous Microgreens

foods

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Article

Ontogenetic Variation in the Mineral, Phytochemical and Yield Attributes of Brassicaceous Microgreens

Marios C. Kyriacou [1], Christophe El-Nakhel [2], Antonio Pannico [2], Giulia Graziani [3], Armando Zarrelli [4], Georgios A. Soteriou [1], Angelos Kyratzis [1], Chrystalla Antoniou[1], Fabiana Pizzolongo [2], Raffaele Romano [2], Alberto Ritieni [3], Stefania De Pascale [2] and Youssef Rouphael [2,*]

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Abstract

Microgreens constitute novel gastronomic ingredients that combine visual, kinesthetic and bioactive qualities. The definition of the optimal developmental stage for harvesting microgreens remains fluid. Their superior phytochemical content against mature leaves underpins the current hypothesis of significant changes in compositional profile during the brief interval of ontogeny from the appearance of the first (S1) to the second true leaf (S2). Microgreens of four brassicaceous genotypes (Komatsuna, Mibuna, Mizuna and Pak Choi) grown under controlled conditions and harvested at S1 and S2 were appraised for fresh and dry yield traits. They were further analyzed for macro- and micromineral content using inductively coupled plasma optical emission spectrometry (ICP-OES), carotenoid content using high-performance liquid chromatography with a diode-array detector (HPLC-DAD), volatile organic compounds using solid-phase microextraction followed by gas chromatography-mass spectrometry (SPME-GC/MS), anthocyanins and polyphenols using liquid chromatography-high resolution-tandem mass spectrometry (LC-MS/MS) with Orbitrap technology and for chlorophyll and ascorbate concentrations, well as antioxidant capacity by spectrophotometry. Analysis of compositional profiles revealed genotype as the principal source of variation for all constituents. The response of mineral and phytochemical composition and of antioxidant capacity to the growth stage was limited and largely genotype-dependent. It is, therefore, questionable whether delaying harvest from S1 to S2

would significantly improve the bioactive value of microgreens while the cost-benefit analysis for this decision must be genotype-specific. Finally, the lower-yielding genotypes (Mizuna and Pak Choi) registered higher relative increase in fresh yield between S1 and S2, compared to the faster-growing and higher-yielding genotypes. Although the optimal harvest stage for specific genotypes must be determined considering the increase in yield against reduction in crop turnover, harvesting at S2 seems advisable for the lower-yielding genotypes.

Keywords: *antioxidants; bioactive value; carotenoids; harvest maturity; isothiocyanates; ontogeny; polyphenols; volatile organic compounds*

1. Introduction

Microgreens have gained a position over the past two decades initially in the upscale gastronomy market and subsequently in the mainstream horticultural supply chain as ingredients of outstanding gastronomic and nutritive value. They combine visual, kinesthetic and bioactive qualities derived from their rich mineral and phytochemical content. The range of genotypes (species, cultivars, landraces) employed for growing microgreens commercially has been rapidly expanding along with the industry, yet the majority of genotypes come from the families *Brassicaceae*, *Asteraceae, Chenopodiaceae, Lamiaceae, Apiaceae, Amarillydaceae, Amaranthaceae* and *Cucurbitaceae* (Kyriacou et al., 2016a).

Microgreens have been shown to be good sources of minerals that constitute necessary dietary intake requirements, particularly of the macroelements (K, Ca and Mg) and the microelements (Fe and Zn; Xiao et al., 2016; Kyriacou et al., 2019a). The phytochemical content of microgreens attributed bioactive value comprises phenolic compounds, encountered as flavonoids (including anthocyanidins) and nonflavonoids (mostly phenolic and hydroxycinnamic acids), carotenoids (*β*-carotene, lutein and violaxanthin), ascorbic acid, phylloquinone and tocopherols (Xiao et al., 2012; Brazaitytė et al., 2015; Kyriacou et al., 2016a, 2020; Di Bella et al., 2020). Microgreens of the *Brassicaceae*, which comprise the most extensive repository of genetic resources for commercial microgreens production (Di Gioia and Santamaria, 2015; Kyriacou et al., 2016a), are also significant sources of glucosinolates (Kopsell and Sams, 2013; Sun et al., 2015), encountered mostly in the form

of isothiocyanates (Marchioni et al., 2021), which indicates an active glucosinolate metabolism in the early post-germination stages of development.

The mineral and phytochemical composition of microgreens and their *in vitro* antioxidant capacity has been found responsive to several preharvest factors, among which the most influential seems to be the choice of genotype (Xiao et al., 2012; Kyriacou et al., 2016a). Growth conditions nonetheless also demonstrate the capacity to modulate the compositional profiles of microgreens significantly. These include substrate material, which may range considerably in physicochemical parameters between natural fiber and synthetic substrates (Di Gioia et al., 2017; Kyriacou et al., 2019a), and nutrient supplementation strategies (Murphy et al., 2010; Hu et al., 2015; Przybysz et al., 2016a, 2016b; Renna et al., 2016). Moreover, pre-harvest light conditions (quality, intensity and photoperiod) have been demonstrated effective tools in modulating growth and compositional attributes of microgreens, particularly secondary metabolites, rendering Light Emitting Diode (LED) light modules instrumental for the operation of microgreen plant factories (Alrifai et al., 2019; Kyriacou et al., 2019c; Brazaitytė et al., 2021).

The developmental stages of the early post-germination plant ontogeny may dictate significant changes in plant physicochemical constitution and phytochemical content (Pinto et al., 2015; Di Bella et al., 2020; El-Nakhel et al., 2020). The optimal stage for harvesting microgreens should combine prime yield attributes and high crop turnover with prime sensory and functional quality. Previous research on microgreens differentiates clearly sprouts from microgreens, based on light requirements during growth and the harvesting of microgreens without roots at the substrate-hypocotyl interface. Yet the definition of microgreens with respect to the exact developmental stage at harvest remains relatively fluid. Kyriacou et al. (2016a) defined microgreens as greens harvested upon the appearance of the first pair of true leaves, when cotyledons are fully expanded and still turgid. However, the developmental stage at harvest reported in different works varies, with some applying harvest at the cotyledonary stage (Waterland et al., 2017; Klopsch et al., 2019), others at the appearance of the first true leaf (Di Gioia and Santamaria, 2015; De la Fuente et al., 2019; Di Bella et al., 2020) and yet others at the second true leaf (Xiao et al., 2014; Waterland et al., 2017; Kyriacou et al., 2019a, 2020). Other than the obvious change in fresh biomass between successive stages of ontogeny, previous work has also demonstrated significant

changes in secondary plant metabolites and mineral content that constitute integral parts of the vegetables' functional qualities (Kyriacou and Rouphael, 2018).

The superior phytochemical content of microgreens against mature leaves postulated in previous works (Xiao et al., 2012; Pinto et al., 2015; Waterland et al., 2017), has underpinned the current hypothesis that significant changes in compositional profile may take place during the brief interval of microgreens' ontogeny from the appearance of the first (S1) to the second true leaf (S2). Accordingly, microgreens of four brassicaceous genotypes (Komatsuna, Mibuna, Mizuna and Pak Choi) grown under controlled conditions and harvested at S1 and S2 were appraised for fresh and dry yield traits. They were further analyzed for macro- and micromineral content by inductively coupled plasma optical emission spectrometry (ICP-OES), carotenoid content through HPLC-DAD, volatile organic compounds using SPME-GC/MS, anthocyanins and polyphenols using Orbitrap LC-MS/MS and chlorophyll and ascorbate concentrations as well as in vitro antioxidant capacity by spectrophotometry. The results of the current study will enhance our understanding of potential ontogenetic variation in the mineral, phytochemical and yield attributes of brassicaceous microgreens and therefore, in the elucidation of microgreens definition with respect to harvest maturity.

2. Materials and Methods

2.1. Microgreen Genotypes and Growth Chamber Conditions

Two green-pigmented genotypes (mibuna (*Brassica rapa* L. subsp. *nipposinica*) and mizuna (*Brassica rapa* var. *japonica* cv. Greens)] and two red-pigmented genotypes [komatsuna (*Brassica rapa* L. var. *perviridis*) and Pak Choi (*Brassica rapa* L. subsp. *chinensis*)] were used. Mizuna seeds were provided by Condor Seed Production (Yuma, Arizona, USA), while the rest of the seeds were bought from CN Seeds Ltd (Pymoor, Ely, Cambrigeshire, UK). The experiment was carried out at the Agricultural Research Institute (Department of Vegetable Crops), Nicosia, Cyprus, in a controlled growth chamber (MIR-554 growth chamber, Panasonic, Gunma, Japan). Seeds were sown at a density of 70000 seeds m^{-2} and germinated in darkness at 24 °C and 100% relative humidity (RH), by applying irrigation with osmotic water until emergence. Upon emergence, the growth chamber was set at 24/18 ± 2 °C and RH at 65/75% according to a 12h photoperiod delivered by Light

Emitting Diode (LED) panels (K5 Series XL750, Kind LED, Santa Rosa, CA, USA). Photosynthetic photon flux density at the canopy level was maintained at 300 ± 10 μmol m^{-2} s^{-1}. Growth was facilitated in plastic trays (W × L × D: 14 × 19 × 6 cm) filled with a peat-based substrate, as described in detail by Pannico et al. (2020). Daily fertigation with a quarter-strength modified Hoagland formulation (electrical conductivity 400 ± 50 mS cm^{-1} and pH 6 ± 0.2) was applied using a laboratory beaker. The composition and concentration of the constituent macro- and microelements is detailed in Pannico et al. (2020). All treatments were replicated three times while daily rotation of tray positions maintained an equal distribution of light and humidity over the chamber growth surface.

2.2. Canopy Colorimetric Measurements, Phenological Harvest Stage and Sampling

Prior to microgreen harvesting, CIELAB color space chroma of the microgreens canopy was measured by means of a portable chroma meter (CR-400, Minolta, Osaka, Japan). Eight measurements per tray/replicate were obtained. Microgreens of the four cultivated Brassica genotypes were harvested at two different growth stages (**Figure 1**): S1 (appearance of the first true leaf) and S2 (appearance of the second true leaf). S1 and S2 in all genotypes were attained 7 and 12 days after sowing, respectively. At each stage, microgreens were harvested just above substrate level and immediately assessed for fresh weight. Afterwards, a random part of the sample was used fresh for the analysis of volatile organic compounds by SPME-GC/MS, another part was conserved at −80 °C for phytochemical analyses (either fresh-frozen or lyophilized), and a part was placed in a forced-air oven at 65 °C until reaching constant dry weight, as determined on an analytical balance (XT120A, Precisa Gravimetrics, Dietikon, Switzerland). Dry matter content was calculated and expressed as percentage of fresh weight. Dried microgreens leaves and stems were ground in a Wiley Mill (841-microns screen) for mineral analysis.

Figure1. Phenological stages for harvesting four *Brassica rapa* L. genotypes (Komatsuna, Mibuna, Mizuna, Pak Choi grown under controlled conditions: S1 - appearance of the first true leaf; S2 - appearance of the second true leaf.

2.3. Chlorophyll, Total Ascorbic Acid and Hydrophilic Antioxidant Capacity

The Lichtenthaler and Wellburn (1983) protocol was implemented for determining the chlorophyll content of microgreens. Spectrophotometric determination of chlorophylls a and b extracted in 90% acetone was performed at 662 and 645 nm on a Hach DR 4000 spectrophotometer (Hach Co, Loveland, CO, USA). Total chlorophyll content was calculated as the sum of chlorophylls a and b and expressed as μg kg^{-1} fw.

Analysis of total ascorbic acid (TAA) was performed according to the method of Kampfenkel et al. (1995) and the results were expressed in mg kg^{-1} fw. The hydrophilic antioxidant capacity (HAC) of lyophilized microgreens was assessed through UV-Vis spectrophotometry based on the N,N-dimethyl-p-phenylenediamine (DMPD) method of Fogliano et al. (1999) and expressed in mmol ascorbate equivalents kg^{-1} dw.

2.4. Analysis of Macro- and Micro-minerals by ICP-OES

Macro (P, K, Ca, Mg, and Na) and microminerals (Fe, Mn, Mo, Se and Zn) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES Spectroblue, Spectro Ametek, Berwyn, PA, USA), as detailed in Volpe et al. (2015). Microwave-assisted digestion (MLS-1200 Microwave Laboratory Systems, Milestone, Shelton, CT, USA) of about 1 g of desiccated microgreens in 10 mL of 65% HNO_3 and 37% HCl (3:1, *v/v*) was performed and the digested solutions were then brought to 50 mL with

ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany). Calibration curves for nonalkaline elements (Fe, Mn, Mo, Se and Zn) were established in the range of standard concentrations from 1.0 to 100 μg L^{-1} and expressed in μg g^{-1} dw. Calibration curves for alkaline elements (P, K, Ca, Mg, and Na) were established in the range of standard concentrations from 100 μg L^{-1} to 10 mg L^{-1} and expressed in mg g^{-1} dw. Accuracy was checked by concurrent analysis of standard reference material (BCR CRM 142R - Commission of the European Communities, 1994) and recoveries ranged from 86 to 98%.

2.5. Analysis of Carotenoids by HPLC-DAD

Carotenoids from lyophilized microgreens samples were extracted, separated and quantified as previously described by Kyriacou et al. (2020) An Agilent HPLC system (Agilent Technologies, Santa Clara, CA, USA) furnished with a 1200 Series quaternary pump and a 1260 Diode Array Detector Separation was employed. Separation was implemented on a Gemini C18 (Phenomenex, Torrance, CA, USA) reverse phase column (250 mm × 4.6 mm, 5 μm). Quantification was based on external standard calibration curves established using β-carotene and lutein commercial standards at six concentrations in the range 5 to 100 μg ml^{-1}. Results were expressed in mg kg^{-1} dw. A representative HPLC-Diode array chromatogram of carotenoids extracted from Komatsuna microgreens monitored at 450 nm is reported in **Figure S1**.

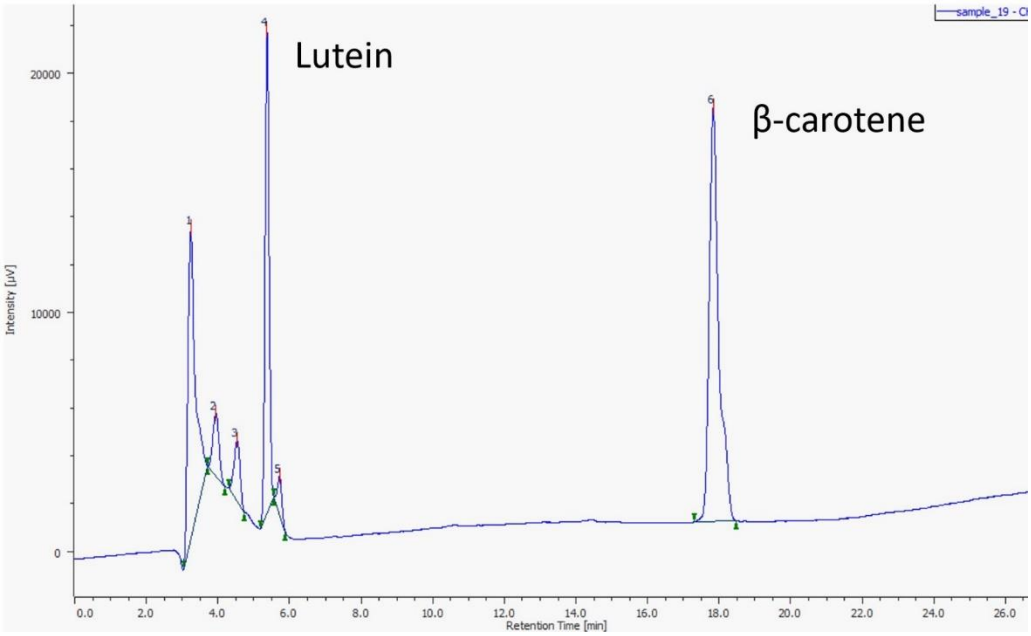


Figure S1. Representative HPLC-Diode array chromatogram of carotenoids extracted from Komatsuna microgreens monitored at 450 nm.

2.6. Analysis of Anthocyanins and Polyphenols by UHPLC-Q-Orbitrap HRMS

Anthocyanins and polyphenols were extracted from lyophilized microgreens and analyzed by the method detailed in Kyriacou et al. (2020) and (El-Nakhel et al., 2021). Analysis was facilitated on a UHPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific, Ma, USA) coupled to a Q-Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fischer Scientific, Ma, USA). Chromatographic separation of anthocyanins and polyphenols was performed using a Luna Omega PS 1.6 µm (50 mm × 2.1 mm, Phenomenex) column. Compound identification and quantification was facilitated using a Q-Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fischer Scientific, Waltham, MA, USA) operated in fast negative/positive ion switching mode (Thermo Scientific, Bremen, Germany). Two scan events (Full scan MS and All ion fragmentation, AIF) were set for all compounds of interest. Analysis and processing of data was performed using software Xcalibur v.3.0.63 (Xcalibur, Thermo Fisher Scientific, Waltham, MA, USA).

The concentrations of all anthocyanin and polyphenol compounds were expressed in µg g^{-1} dw. Representative UHPLC-HRMS chromatograms of anthocyanin and polyphenol compounds extracted from Komatsuna microgreens are reported in **Figures S2** and **S3**.

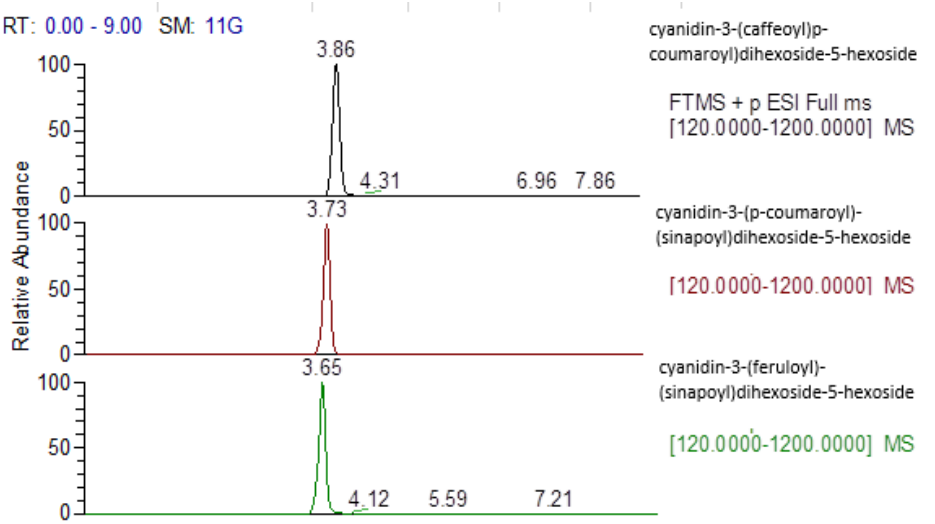


Figure S2. UHPLC-HRMS chromatogram of anthocyanin extracted from Komatsuna microgreens.

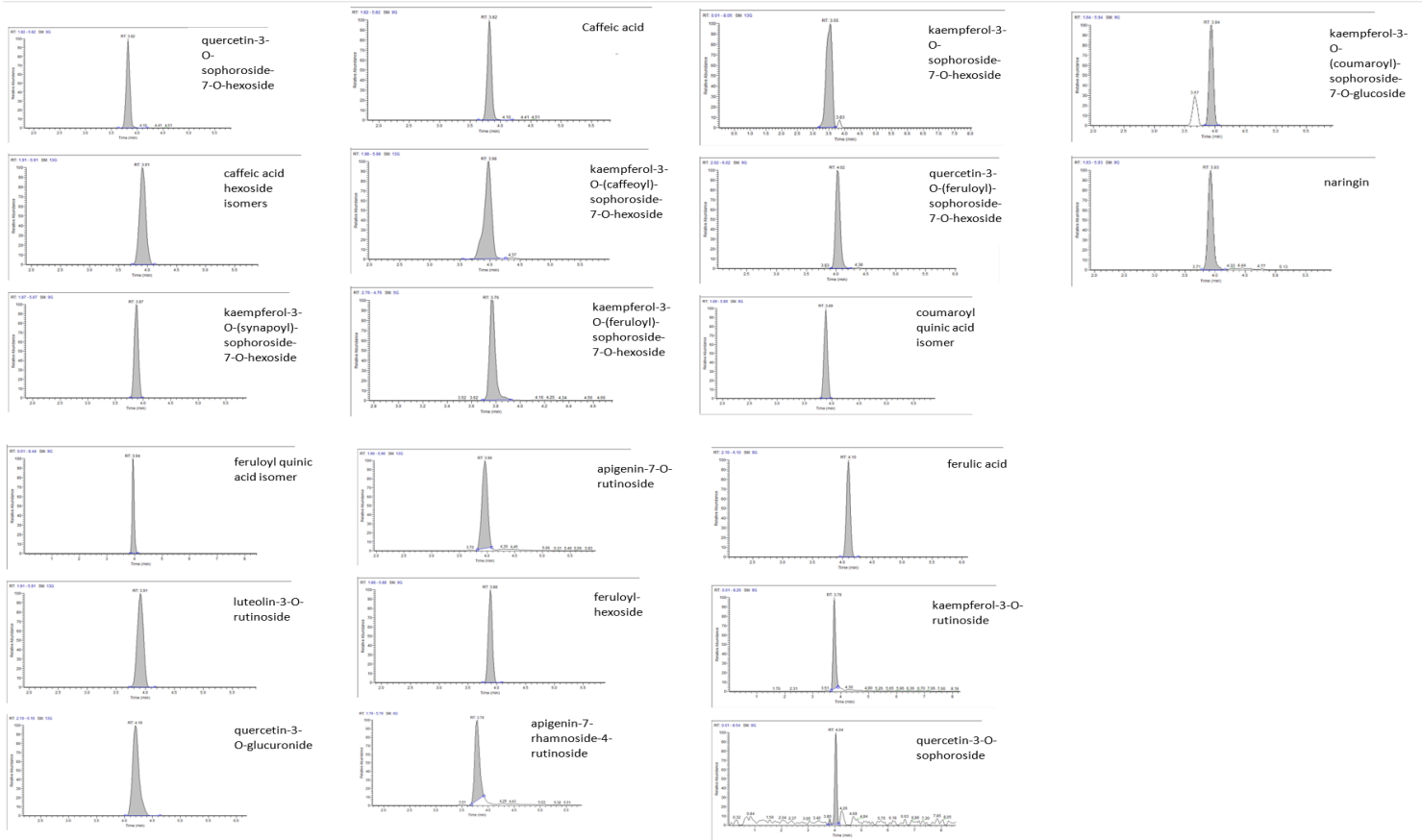


Figure S3. UHPLC-HRMS chromatogram of polyphenolic compounds extracted from Komatsuna microgreens.

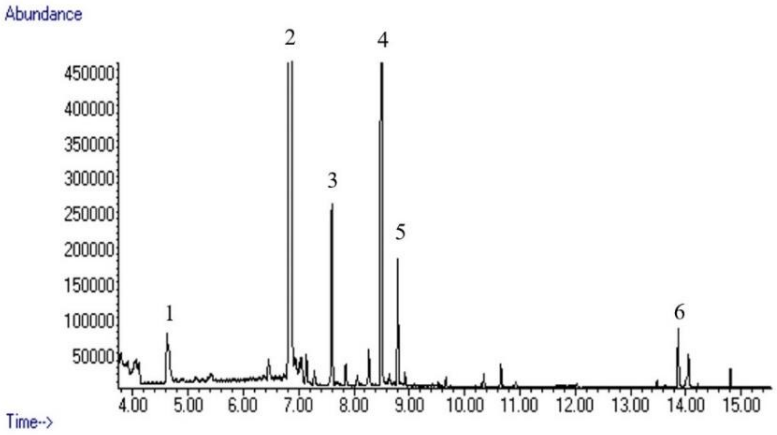
The individual phenolic compounds were identified and quantified by comparison with available standards. In particular, anthocyanins were quantified using an external calibration curve built with cyanidin-glucoside and their concentration was expressed as cyanidin-glucoside equivalents. As regards polyphenolic compounds the external calibration curves of the following standards have been used: rutin for all quercetin derivatives, kaempferol-3-O-glucoside for all kaempferol derivatives, caffeic acid for caffeic acid and its derivatives, ferulic acid for ferulic acid and its derivatives, apigenin-7-O-rutinoside for apigenin-7-O-rutinoside and its derivative, naringin for naringin and finally luteolin-3-O-rutinoside reference standard for luteolin-3-O-rutinoside. The robustness of the analytical method was assessed using the corresponding standards. As some standards were not available for identification MS/MS experiments had to be used. Linearity, the limit of detection (LOD), limit of quantification (LOQ), precision and recovery for the eight authentic standards were reported in supplementary material (**Table S1**).

Table S1. Linearity, limit of detection (LOD), limit of quantification (LOQ), precision and recovery for the 8 authentic standards (n = 5).

Compound	LOQ	LOD	Linearity (r2)	Recovery (%) (n = 3)			Intra-Day Precision (RSD, %; (n = 3)	Inter-Day Precision (RSD, %; (n = 3)
	(ng/g)	(ng/g)		1 mg*kg^{-1}	10 mg*kg^{-1}	50 mg*kg^{-1}	5	6
Cyanidin-glucoside	0.04	0.12	0.998	91.5	92.4	99.3	3	8
Rutin	0.05	0.14	0.976	93.6	94.6	91.4	6	9
Kaempferol-3-O-glucoside	0.04	0.12	0.999	93.4	90.1	89.2	7	10
Caffeic acid	0.05	0.14	0.982	96.6	96.9	98.6	5	5
Ferulic acid	0.03	0.10	0.991	98.5	93.7	92.7	4	7
Apigenin-rutinoside	0.05	0.14	0.993	99.5	98.3	98.5	2	8
Naringin	0.04	0.12	0.999	95.9	95.8	98.4	7	9
Luteolin-3-O-rutinoside	0.03	0.10	0.993	98.3	89.4	93.5	5	7

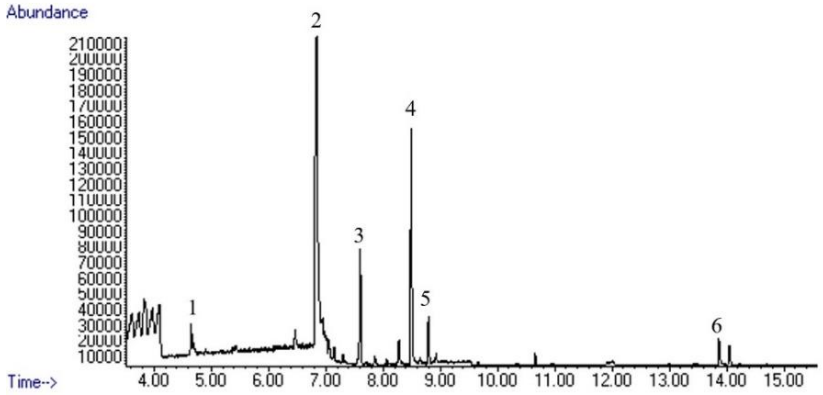
2.7. Analysis of Volatile Organic Compounds by SPME-GC/MS

Volatile organic compounds (VOCs) were extracted by Solid-Phase Micro-Extraction (SPME) and analyzed by Gas Chromatography coupled to Mass Spectrometry (GC/MS), following the methodology outlined by Huang et al. (2011) and Klimánková et al. (2008), with some modifications. One g of fresh microgreens was positioned in a 20 mL headspace glass vial with a screw-top PTFE septum (Supelco®, Bellefonte, Pennsylvania, USA). Migration of the VOCs to headspace was induced by 10 min stirring at 40 °C with an ARE® magnetic stirrer (Velp® Scientifica, Usmate Velate, Monza Brianza, Italy). Adsorption of VOCs was implemented by introducing a 50/30-µm-thick, one-cm-long divinylbenzene/carboxane/polydimethylsiloxane fiber into the vial (Supelco®, Bellefonte, Pennsylvania, USA) for 20 minutes at 40 °C. The fiber was subsequently injected for desorption into a split-splitless injector at 230 °C for 10 minutes of an Agilent 6890N GC coupled to a 5973N MS detector (Agilent, Santa Clara, California, USA). Following a splitless injection of the samples, separation was facilitated in a capillary column 30-m-long and 0.250-mm-thick, coated with a 0.25-µm film of 5% phenyl/ 95% dimethylpolysiloxane (Supelco®, Bellefonte, Pennsylvania, USA). Oven temperature was kept at 50 °C for 2 min, then ramped from 50 to 150 °C at 10 °C min^{-1} and from 150 to 280 °C at 15 °C min^{-1}. Helium was used as the carrier gas at 1 mL min^{-1}. Injection source and ion source temperatures were 250 and 230 °C, respectively, and the MS was operated at 70 eV. Headspace VOCs were analyzed by comparing mass spectra and retention times with the Atomic Spectra Database version 1.6 of National Institute of Standards and Technology (NIST) libraries. Samples were analyzed in triplicates and the results were expressed in percentage (%). Representative chromatograms of VOCs extracted by SPME and analyzed by GC/MS, from Komatsuna and Pack Choi microgreens are reported in **Figure S4**.



Sample: Komatsuna

Peak	Compound
1	trans-2-Hexenal
2	3-Butenyl isothiocyanate
3	Limonene
4	Allyl Isothiocyanate
5	Nonanal
6	Phenethyl isothiocyanate



Sample: Pak choi

Peak	Compound
1	trans-2-Hexenal
2	3-Butenyl isothiocyanate
3	Limonene
4	Allyl Isothiocyanate
5	Nonanal
6	Phenethyl isothiocyanate

Figure S4. Representative chromatograms of VOCs extracted from Komatsuna and Pack Choi microgreens

2.8. Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) using JMP statistical package (SAS Institute, Inc., Cary, NC, USA). Genotype means and genotype × growth stage means were compared and separated according to the Tukey–Kramer honestly significant difference (HSD) test. Growth stage means were compared according to the Student's t-test, provided that the main effect of the growth stage was significant according to the ANOVA.

3. Results

3.1. Yield, Dry Matter and Colorimetric Components

The highest fresh yield was obtained from Mibuna and Komatsuna microgreens and the lowest from Mizuna (**Table 1**). A significant increase in fresh yield was observed between growth stages in all genotypes. However, the relative increase in fresh yield between S1 and S2 differed markedly between genotypes, as indicated by significant M × S interaction. Intriguingly, the largest increase was noted in Mizuna (80.1%) and Pak Choi (33.3%), which were the genotypes having lower mean yield than the Mibuna and Komatsuna microgreens. Similar effects were observed for dry weight with significantly higher dry weight obtained for Mibuna and Komatsuna than Pak Choi and Mizuna, with the latter incurring however the largest increase (119%) between growth stages. Pak Choi and Mizuna also attained lower dry matter content than Mibuna and Komatsuna. Dry matter increased from S1 to S2 by a mean increment of 12.2% in all genotypes except Mibuna, which did not incur significant change in dry matter content. Chroma was most intense in the green-colored Mibuna and Mizuna compared to the darker-colored Komatsuna and Pak Choi. Chroma increased between S1 and S2 in all genotypes but higher relative increase was observed in the purple-colored than the green-colored genotypes.

Table1. Yield and colorimetric attributes of four *Brassica rapa* L. microgreens genotypes harvested at the first (S1) or second true-leaf stage (S2). All data are expressed as mean ± standard error, $n = 3$.

Source of variance	Yield (kg FW m^{-2})	Dry weight (g m^{-2})	Dry matter (%)	Chroma
Genotype (M)				
Komatsuna	2.65 ± 0.14 a	157 ± 14 a	5.89 ± 0.21	4.61 ± 0.33
Mibuna	2.63 ± 0.16 a	165 ± 12 a	6.27 ± 0.09	23.58 ± 0.94
Mizuna	2.19 ± 0.28 c	120 ± 20 c	5.35 ± 0.24	33.29 ± 1.05
Pak choi	2.45 ± 0.16 b	136 ± 11 b	5.54 ± 0.12	4.26 ± 0.79
p-value	<0.001	<0.001	<0.001	<0.001
Growth stage (S)				
S1	2.07 ± 0.10	113 ± 7.5	5.43 ± 0.15	14.74 ± 3.61
S2	2.89 ± 0.03	176 ± 4.6	6.09 ± 0.09	18.13 ± 3.92
p-value	<0.001	<0.001	<0.001	0.051
M × S				
Komatsuna × S1	2.34 ± 0.05 b	127 ± 4.5 c	5.42 ± 0.08	3.96 ± 0.04
Komatsuna × S2	2.95 ± 0.09 a	188 ± 7.1 a	6.35 ± 0.06	5.26 ± 0.33 f
Mibuna × S1	2.27 ± 0.08 bc	140 ± 4.5 c	6.17 ± 0.03	21.52 ± 0.16
Mibuna × S2	2.99 ± 0.03 a	190 ± 6.3 a	6.37 ± 0.17	25.65 ± 0.34
Mizuna × S1	1.56 ± 0.08 d	75.2 ± 4.6 e	4.82 ± 0.06	30.95 ± 0.15
Mizuna × S2	2.81 ± 0.06 a	165 ± 6.1 b	5.88 ± 0.10	35.64 ± 0.17
Pak choi × S1	2.10 ± 0.04 c	111 ± 3.0 d	5.31 ± 0.11	2.54 ± 0.19
Pak choi × S2	2.80 ± 0.03 a	161 ± 1.4 b	5.77 ± 0.08	5.98 ± 0.29
p-value	<0.001	0.005	0.002	<0.001

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$). Microgreens growth stages were compared according to Student's *t*-test.

3.2. Mineral Composition

All eleven macro- and microminerals examined differed significantly between genotypes (**Table 2**). Pak Choi and Komatsuna were the microgreen genotypes with the highest content in the macrominerals P and K. Pak Choi was also the genotype richest in the macromineral Mg and the microminerals Fe and Zn. Mibuna was the richest genotype in Ca and in the microminerals Mn and Se, whereas Mizuna was the richest in Na and Mo.

Phosphorous, Na and Mo concentrations did not differ between growth stages in any of the genotypes examined. Potassium, Ca, Mg, Fe, Se and Zn concentrations exhibited a variable response to the growth stage as indicated by significant M × S interaction. Potassium concentration did not differ between stages in Mibuna, Mizuna and Pak Choi,

whereas in Komatsuna concentration was lower at S2 compared to S1. Calcium concentration was overall higher at S2 than S1. However, in Mizuna and Komatsuna, this difference was nonsignificant. Magnesium concentration was unchanged between growth stages in Komatsuna, Mizuna and Pak Choi but it increased significantly at S2 in Mibuna. Iron concentration exhibited the most varied response across genotypes with higher concentration observed in Komatsuna and Pak Choi at S1, lower concentration at S1 in Mibuna and no change in Mizuna. Selenium concentration showed no response to the growth stage in all genotypes but Pak Choi demonstrated higher concentration at S1. Finally, Zn concentration increased at S2 in Mizuna but remained unaltered in the rest three genotypes (**Table 2**).

In summary, major changes between growth stages were observed for macrominerals K, Ca and Mg and microminerals Fe and Mn. Decrease from S1 to S2 was the prevalent change for the concentrations of K and Fe as opposed to an increase for the concentrations of Ca, Mg, and Mn. Except for Ca, however, these responses were not uniform across genotypes. On the other hand, the concentrations of P, Na, Mo and to lesser degree those of Se and Zn were nonresponsive to the growth stage.

Table 2. Mineral content of four *Brassica rapa* L. microgreens genotypes harvested at the first (S1) or second true-leaf stage (S2). All data are expressed as mean ± standard error, $n = 3$.

Source of variance	P (mg g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Mg (mg g^{-1} DW)	Na (mg g^{-1} DW)	Fe (µg g^{-1} DW)	Mn (µg g^{-1} DW)	Mo (µg g^{-1} DW)	Se (µg g^{-1} DW)	Zn (µg g^{-1} DW)
Genotype (M)										
Komatsuna	10.30 ± 0.2 a	16.83 ± 0.6 a	10.28 ± 0.4 c	4.71 ± 0.1 ab	2.75 ± 0.1 b	134 ± 5.2 b	189 ± 19.6 b	8.05 ± 0.3 b	2.32 ± 0.1 ab	102 ± 1.8 bc
Mibuna	7.64 ± 0.2 b	11.92 ± 0.4 b	12.38 ± 0.4 a	4.52 ± 0.2 b	2.04 ± 0.1 c	138 ± 6.7 b	233 ± 29.3 a	10.33 ± 0.4 a	2.73 ± 0.1 a	108 ± 3.0 b
Mizuna	7.16 ± 0.2 b	12.80 ± 0.4 b	11.79 ± 0.2 ab	4.35 ± 0.1 b	3.22 ± 0.2 a	115 ± 2.5 c	194 ± 23.6 b	11.72 ± 0.8 a	2.53 ± 0.1 ab	96 ± 5.1 c
Pak choi	10.58 ± 0.2 a	17.08 ± 0.4 a	11.28 ± 0.6 b	5.05 ± 0.1 a	2.78 ± 0.1 b	180 ± 6.3 a	210 ± 28.1 ab	10.19 ± 0.6 a	2.02 ± 0.3 b	141 ± 2.9 a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	<0.001
Growth stage (S)										
S1	9.02 ± 0.4	15.23 ± 0.8	10.70 ± 0.3 [b]	4.43 ± 0.1 [b]	2.89 ± 0.2	142 ± 9.3	152 ± 4.0 [b]	10.70 ± 0.6	2.54 ± 0.1	109 ± 6.7
S2	8.82 ± 0.5	14.09 ± 0.7	12.17 ± 0.3 [a]	4.88 ± 0.1 [a]	2.51 ± 0.1	142 ± 6.5	261 ± 9.6 [a]	9.44 ± 0.4	2.26 ± 0.1	115 ± 4.5
p-value	0.338	0.057	0.002	0.002	0.051	0.920	<0.001	0.054	0.168	0.120
M × S										
Komatsuna × S1	10.51 ± 0.1	18.19 ± 0.1 a	9.72 ± 0.4 d	4.64 ± 0.1 bcd	2.98 ± 0.0	143 ± 6.1 cd	146 ± 5.9	8.16 ± 0.3	2.28 ± 0.1 ab	100 ± 1.1 bc
Komatsuna ×S2	10.08 ± 0.3	15.48 ± 0.2 bc	10.83 ± 0.5 cd	4.78 ± 0.1 abc	2.53 ± 0.1	125 ± 4.9 de	232 ± 3.2	7.93 ± 0.6	2.36 ± 0.1 a	103 ± 3.4 b
Mibuna × S1	7.58 ± 0.3	12.08 ± 0.4 d	11.58 ± 0.1 bc	4.05 ± 0.1 d	2.11 ± 0.1	123 ± 1.3 de	172 ± 6.3	10.29 ± 0.7	2.86 ± 0.1 a	106 ± 5.9 b
Mibuna × S2	7.70 ± 0.2	11.75 ± 0.7 d	13.19 ± 0.3 a	4.98 ± 0.1 ab	1.97 ± 0.1	153 ± 0.8 bc	295 ± 21.6	10.38 ± 0.5	2.61 ± 0.1 a	110 ± 2.6 b
Mizuna × S1	7.53 ± 0.1	13.59 ± 0.4 cd	11.56 ± 0.3 bc	4.24 ± 0.1 cd	3.55 ± 0.2	111 ± 1.5 e	143 ± 4.1	13.15 ± 0.9	2.47 ± 0.1 a	85 ± 3.4 c
Mizuna × S2	6.79 ± 0.1	12.00 ± 0.4 d	12.03 ± 0.2 abc	4.47 ± 0.2 bcd	2.89 ± 0.2	120 ± 2.8 de	245 ± 13.9	10.29 ± 0.4	2.58 ± 0.1 a	106 ± 2.3 b
Pak choi × S1	10.45 ± 0.2	17.05 ± 0.7 ab	9.93 ± 0.2 d	4.80 ± 0.2 abc	2.91 ± 0.2	190 ± 8.4 a	149 ± 3.0	11.22 ± 0.5	2.57 ± 0.4 a	143 ± 4.1 a
Pak choi × S2	10.72 ± 0.2	17.11 ± 0.6 ab	12.64 ± 0.2 ab	5.30 ± 0.1 a	2.65 ± 0.1	171 ± 6.1 ab	272 ± 12.6	9.16 ± 0.5	1.47 ± 0.2 b	139 ± 4.7 a
p-value	0.125	0.008	0.015	0.040	0.200	<0.001	0.128	0.970	0.023	0.012

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$). Microgreens growth stages were compared according to Student's *t*-test.

3.3. Antioxidant Capacity, Ascorbic Acid, Chlorophyll, Lutein and β-carotene

The hydrophilic antioxidant capacity (HAC) of the aqueous extract differed significantly between the four microgreen genotypes studied (**Table 3**). The HAC was highest in Pak Choi and Komatsuna and lowest in Mizuna. The HAC did not change between growth stages in Mibuna and Pak Choi but was found higher at S1 in Komatsuna and Mizuna. Ascorbic acid concentration also differed significantly between genotypes, being highest in Mizuna and lowest in Pak Choi. Ascorbic acid concentration did not change between growth stages in Mibuna, increased from S1 to S2 in Komatsuna and Mizuna, whereas it decreased from S1 to S2 in Pak Choi. Total chlorophyll content was highest in Komatsuna and Pak Choi, which were additionally the two genotypes of microgreens that demonstrated no change in chlorophyll content between growth stages (**Table 3**). On the contrary, Mibuna and Mizuna were the two genotypes of microgreens that incurred significant decrease in chlorophyll content from S1 to S2. In all four genotypes, the principal carotenoid concentration was that of *β*-carotene over lutein, with the former being about two-fold higher than the latter across genotypes (**Table 3**). Lutein was highest in Mibuna and lowest in Mizuna. Komatsuna, Mizuna and Pak Choi showed no response to the growth stage for lutein concentration whereas Mibuna incurred increased concentrations at S2. Finally, *β*-carotene was highest in Mibuna and lowest in Mizuna, thus corresponding to the inter-specific differences also observed for lutein. However, *β*-carotene concentration remained stable between growth stages in all four genotypes of microgreens (**Table 3**).

Table 3. Hydrophillic Antioxidant Capacity (HAC), ascorbic acid, total chlorophyll, lutein and *β*-carotene contents of four *Brassica rapa* L. microgreens harvested at the first (S1) or second true-leaf stage (S2). All data are expressed as mean ± standard error, $n = 3$.

Source of variance	HAC (mmol ascorbate eq. kg^{-1} DW)	Ascorbic acid (mg kg^{-1} FW)	Total chlorophyll (μg kg^{-1} FW)	Lutein (mg kg^{-1} DW)	*β*-carotene (mg kg^{-1} DW)
Genotype (M)					
Komatsuna	26.07 ± 0.8 a	106.2 ± 5.3 b	953 ± 22.3 a	195.8 ± 3.5 b	461.0 ± 9.3 b
Mibuna	23.41 ± 0.2 b	104.0 ± 1.7 b	567 ± 43.8 b	251.1 ± 19.6 a	502.7 ± 15.0 a
Mizuna	21.07 ± 1.3 c	119.3 ± 4.8 a	551 ± 22.7 b	124.6 ± 6.7 c	308.9 ± 10.3 c
Pak choi	26.41 ± 0.4 a	73.8 ± 10.6 c	960 ± 19.3 a	203.1 ± 8.3 b	339.5 ± 9.7 c
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
Growth stage (S)					
S1	25.07 ± 0.6	102.7 ± 1.9	799 ± 51.8	177.9 ± 10.8	388.8 ± 25.6
S2	23.40 ± 1.0	98.9 ± 9.2	717 ± 70.4	209.3 ± 18.0	417.2 ± 24.8
p-value	0.070	0.662	0.052	0.065	0.055
M × S					
Komatsuna × S1	27.45 ± 1.0 a	96.8 ± 1.4 c	956 ± 28.0 a	192.4 ± 6.2 b	461.4 ± 19.7
Komatsuna × S2	24.69 ± 0.4 bc	115.6 ± 7.2 ab	949 ± 41.0 a	199.1 ± 3.3 b	460.5 ± 6.8
Mibuna × S1	23.21 ± 0.4 c	107.6 ± 1.0 bc	665 ± 2.4 b	211.8 ± 5.4 b	478.5 ± 17.0
Mibuna × S2	23.61 ± 0.2 c	100.3 ± 0.6 bc	470 ± 7.7 d	290.3 ± 18.6 a	526.9 ± 15.9
Mizuna × S1	23.74 ± 1.0 c	109.3 ± 1.8 bc	598 ± 7.7 bc	120.9 ± 11.5 c	293.9 ± 17.1
Mizuna × S2	18.39 ± 0.9 d	129.3 ± 3.3 a	503 ± 15.9 cd	128.2 ± 9.0 c	323.8 ± 4.2
Pak choi × S1	25.89 ± 0.4 ab	97.1 ± 2.7 c	974 ± 36.6 a	186.6 ± 4.7 b	321.4 ± 8.1
Pak choi × S2	26.93 ± 0.5 a	50.6 ± 3.4 d	946 ± 18.1 a	219.5 ± 7.0 b	357.5 ± 9.0
p-value	<0.001	<0.001	0.009	0.007	0.269

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$).

3.4. Volatile Aromatic Compounds

A total of 18 volatile aromatic compounds (VOCs) were identified in the four microgreen genotypes at the first and second true leaf stages, six of which accounted for more than 90% of the total abundance (total peak area) and each demonstrated a relative abundance above 1% (**Table 4**). The most abundant VOCs in all four genotypes were the isothiocyanate compounds 3-butenyl isothiocyanate, allyl isothiocyanate and phenethyl isothiocyanate, which accounted for >85% of the VOCs relative abundance. These were succeeded in relative abundance by the aldehydes trans-2-hexenal and nonanal, and the cyclic monoterpene limonene. The relative abundance of all eight major VOCs presented was significantly affected by genotype but presented a variable response to the growth stage, as indicated by significant M × S interaction.

The highest relative abundance in 3-butenyl isothiocyanate was observed in Mibuna and the lowest in Mizuna; moreover, it was found in higher relative abundance in stage S1 microgreens of all genotypes, except from Mizuna, which demonstrated no change in response to the growth stage (**Table 4**). A reverse motif was observed for allyl isothiocyanate that was most abundant in Mizuna and least in Mibuna. As opposed to the trend observed for 3-butenyl isothiocyanate, the relative abundance of allyl isothiocyanate increased at S2 in Komatsuna and marginally in Pak Choi but remained nonresponsive to the growth stage in Mibuna and Mizuna microgreens. Pak Choi and Mizuna microgreens had the highest relative abundance of phenethyl isothiocyanate and Mibuna the lowest. In Mizuna and Pak Choi microgreens, the presence of phenethyl isothiocyanate decreased marginally at S2 whereas in Komatsuna it increased and in Mibuna change was nonsignificant (**Table 4**). Limonene was found in highest relative abundance in Komatsuna and in lowest in Mizuna microgreens. In Mibuna and Pak Choi it increased at S2, whereas Komatsuna and Mizuna

demonstrated no change in response to the growth stage. Nonanal was highest in Pak Choi and increased significantly at S2 in Mibuna and Pak Choi whereas in Komatsuna and Mizuna the increase was nonsignificant. The relative abundance of nonanal with response to the growth stage did not change significantly in Komatsuna and Mizuna whereas it increased at S2 in Mibuna and Pak Choi microgreens.

Finally, trans-2-hexenal was found in highest relative abundance in Pak Choi and in lowest in Mibuna and Mizuna; while it increased at S2 in Pak Choi and Komatsuna, changes the in Mibuna and Mizuna microgreens were nonsignificant (**Table 4**). Overall, variation in the relative abundance of the major VOCs identified was higher between genotypes than growth stages, with response to the growth stage also varying between genotypes. The most common response to the growth stage was either increase at S2 or no change. It is worth noting however that the most prevalent volatile aromatic component in the microgreens profile (3-butenyl isothiocyanate) had higher relative abundance at S1 than S2, whereas the reverse trend was dominant for the rest of the identified VOCs.

Table 4. Volatile Organic Compounds (VOCs) content of four *Brassica rapa* L. microgreens harvested at the first (S1) or second true-leaf stage (S2). All data are expressed as mean ± standard error, n = 3. All data are expressed as mean ± standard error, *n* = 3.

Source of variance	trans-2-Hexenal	3-Butenyl isothiocyanate	Limonene	Allyl isothiocyanate	Nonanal	Phenethyl isothiocyanate
	Relative percentage content (%)					
Genotype (M)						
Komatsuna	1.26 ± 0.37 b	78.17 ± 2.02 b	5.03 ± 0.66 a	10.83 ± 1.13 b	1.48 ± 0.17 b	0.89 ± 0.19 bc
Mibuna	0.11 ± 0.05 c	88.45 ± 1.68 a	2.18 ± 0.97 bc	5.16 ± 0.44 d	1.84 ± 0.56 b	0.38 ± 0.13 c
Mizuna	0.53 ± 0.19 c	68.22 ± 0.60 c	0.70 ± 0.30 c	26.18 ± 0.42 a	1.12 ± 0.23 b	1.64 ± 0.25 a
Pak choi	2.81 ± 1.12 a	76.38 ± 3.54 b	2.69 ± 0.75 b	7.29 ± 0.69 c	3.36 ± 0.77 a	1.42 ± 0.31 ab
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Growth stage (S)						
S1	0.25 ± 0.06 b	81.84 ± 2.51 a	1.45 ± 0.50 b	11.49 ± 2.54	1.10 ± 0.14 b	1.31 ± 0.25
S2	2.11 ± 0.59 a	73.77 ± 2.15 b b	3.85 ± 0.62 a	13.24 ± 2.49	2.80 ± 0.45 a	0.85 ± 0.14
p-value	0.005	0.023	0.006	0.626	0.002	0.123
M × S						
Komatsuna × S1	0.46 ± 0.08 c	82.63 ± 0.12 b	4.05 ± 0.76 a	8.54 ± 0.61 c	1.25 ± 0.19 c	0.65 ± 0.12 b
Komatsuna × S2	2.06 ± 0.15 b	73.71 ± 0.67 c	6.01 ± 0.81 a	13.11 ± 0.86 b	1.72 ± 0.24 bc	1.13 ± 0.33 ab
Mibuna × S1	0.01 ± 0.01 c	91.77 ± 0.83 a	0.19 ± 0.02 b	5.39 ± 0.41 cd	0.76 ± 0.15 c	0.57 ± 0.22 b
Mibuna × S2	0.21 ± 0.05 c	85.13 ± 1.57 b	4.17 ± 0.86 a	4.94 ± 0.87 d	2.92 ± 0.59 b	0.18 ± 0.04 b
Mizuna × S1	0.12 ± 0.03 c	68.78 ± 0.59 d	0.44 ± 0.33 b	25.93 ± 0.23 a	0.73 ± 0.16 c	2.13 ± 0.26 a
Mizuna × S2	0.94 ± 0.08 c	67.65 ± 1.06 d	0.96 ± 0.53 b	26.44 ± 0.87 a	1.51 ± 0.30 bc	1.16 ± 0.08 ab
Pak choi × S1	0.39 ± 0.05 c	84.17 ± 0.69 b	1.11 ± 0.22 b	6.08 ± 0.14 cd	1.66 ± 0.24 bc	1.91 ± 0.48 a
Pak choi × S2	5.23 ± 0.60 a	68.58 ± 1.16 d	4.27 ± 0.49 a	8.49 ± 0.96 c	5.06 ± 0.16 a	0.93 ± 0.15 ab
p-value	<0.001	<0.001	0.048	0.006	<0.001	0.015

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$). Microgreens growth stages were compared according to Student's *t*-test.

3.5. Anthocyanins

Variation in the total concentration of anthocyanins present in the four microgreens genotypes was wide and corresponded distinctly to their visual pigmentation (**Table 5**). In this respect, total anthocyanins were more concentrated in the purple-leaf genotypes of Pak Choi and Komatsuna and significantly less concentrated in the green-leaf genotypes of Mibuna and Mizuna. The most abundant anthocyanin component in all genotypes was cyanidin-3-(feruloyl)(sinapoyl)dihexoside-5-hexoside, which largely defined genotype behavior in response to the growth stage. The effect of the growth stage on all anthocyanin components examined was nonsignificant (**Table 5**). However, interspecific differences were observed in response to the growth stage, as highlighted by significant interaction. In the green-leaf genotypes of Mibuna and Mizuna differences between S1 and S2 were nonsignificant for all anthocyanin components, as well as total anthocyanins. For the purple-leaf genotypes of Komatsuna and Pak Choi opposite responses to the growth stage were observed. In Komatsuna, cyanidin-3-(feruloyl)(sinapoyl)dihexoside-5-hexoside, cyanidin-3-(p-coumaroyl)(sinapoyl)dihexoside-5-hexoside as well as total anthocyanins increased at S2 and only cyanidin-3-(caffeoyl)(p-coumaroyl)dihexoside-5-hexoside decreased. In Pak Choi by contrast, cyanidin-3-(feruloyl)(sinapoyl)dihexoside-5-hexoside and total anthocyanins were higher at S1 than S2, while the reverse trend was observed for cyanidin-3-(caffeoyl)(p-coumaroyl)dihexoside-5-hexoside and cyanidin-3-(p-coumaroyl)(sinapoyl)dihexoside-5-hexoside (**Table 5**). In summary, anthocyanin concentration reflected closely the visual pigmentation of the microgreen species and no response to the growth stage was seen in green-leaf microgreens. Whereas in the case of purple-leaf genotypes response to growth space was genotype-specific with respect to individual anthocyanin components and total anthocyanin content.

Table 5: Anthocyanins content of four *Brassica rapa* L. microgreens harvested at the first (S1) or second true-leaf stage (S2). All data are expressed as mean ± standard error, n = 3. All data are expressed as mean ± standard error, *n* = 3.

Source of variance	cyanidin-3-(caffeoyl)(p-coumaroyl)dihexoside-5-hexoside (µg g^{-1} DW)	cyanidin-3-(p-coumaroyl)(sinapoyl)dihexoside-5-hexoside (µg g^{-1} DW)	cyanidin-3-(feruloyl)(sinapoyl)dihexoside-5-hexoside (µg g^{-1} DW)	∑ Anthocyanins (µg g^{-1} DW)
Genotype (M)				
Komatsuna	0.43 ± 0.028 b	1.98 ± 0.277 b	8.00 ± 1.22 b	10.41 ± 1.456 b
Mibuna	0.05 ± 0.003 c	0.35 ± 0.024 c	1.96 ± 0.07 c	2.36 ± 0.056 c
Mizuna	0.09 ± 0.008 c	0.07 ± 0.019 c	0.76 ± 0.10 c	0.93 ± 0.116 c
Pak choi	0.56 ± 0.033 a	4.86 ± 0.382 a	12.79 ± 1.40 a	18.21 ± 1.150 a
p-value	<0.001	<0.001	<0.001	<0.001
Growth stage (S)				
S1	0.28 ± 0.065	1.47 ± 0.478	5.84 ± 1.79	7.59 ± 2.315
S2	0.28 ± 0.070	2.17 ± 0.677	5.92 ± 1.36	8.37 ± 2.031
p-value	0.947	0.606	0.951	0.538
M × S				
Komatsuna × S1	0.49 ± 0.004 b	1.39 ± 0.137 d	5.30 ± 0.08 c	7.17 ± 0.152 c
Komatsuna × S2	0.37 ± 0.012 c	2.58 ± 0.074 c	10.70 ± 0.31 b	13.66 ± 0.241 b
Mibuna × S1	0.04 ± 0.002 d	0.39 ± 0.027 e	1.90 ± 0.02 d	2.33 ± 0.006 d
Mibuna × S2	0.05 ± 0.001 d	0.31 ± 0.014 e	2.02 ± 0.13 d	2.38 ± 0.122 d
Mizuna × S1	0.09 ± 0.007 d	0.03 ± 0.001 e	0.55 ± 0.06 d	0.67 ± 0.064 d
Mizuna × S2	0.09 ± 0.016 d	0.11 ± 0.004 e	0.97 ± 0.03 d	1.18 ± 0.014 d
Pak choi × S1	0.50 ± 0.026 b	4.06 ± 0.208 b	15.61 ± 0.75 a	20.17 ± 0.956 a
Pak choi × S2	0.62 ± 0.035 a	5.66 ± 0.207 a	9.97 ± 1.13 b	16.26 ± 1.365 b
p-value	<0.001	<0.001	<0.001	<0.001

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$).

3.6. Polyphenols

Twenty polyphenols were identified and quantified by UHPLC-Q-Orbitrap HRMS in the four microgreen genotypes at S1 and S2 (**Table 6**). Genotype effect on all polyphenols and total phenolic content was significant. Growth stage effect was significant most of the phenolic components quantified and the total phenolic content of microgreens with a prevalent trend for increase at S2. However, variation in phenolic content was primarily defined by genotype. Interspecific variation in total phenolic content was wide (630 – 1933 μg g^{-1} dw), with the highest content observed in Mizuna and the lowest in Komatsuna microgreens (**Table 6**). Despite differences in total phenolic content however, the four microgreen genotypes presented relatively similar compositional profiles of polyphenols. As a fraction of the total phenolic content across genotypes, flavonol glycosides represented 71.2 − 82.3 %, hydroxycinnamic acids and their derivatives 16.4 – 26.1 % and flavones 1.3 – 2.7 %.

In all genotypes, the most abundant phenolic compounds in declining order were: kaempferol-3-*O*-(synapoyl)-sophoroside-7-*O*-hexoside, quercetin-3-*O*-glucuronide, feruloyl glycoside and caffeic acid hexoside (**Table 6**). The total phenolic content of microgreens and the phenolic components responsive to the growth stage were higher at S2 than S1. For instance, ferulic acid and feruloyl glycoside concentrations increased in all genotypes from S1 to S2. Therefore, a trend for increasing concentration with transition from S1 to S2 was discerned for most phenolic constituents and the total phenolic content (**Table 6**).

Table 6. Phenolic composition of four Brassica rapa L. microgreens harvested at the first (S1) or second true-leaf stage (S2). All data are expressed as mean ± standard error, $n = 3$.

Source of variance	quercetin-3-*O*-sophoroside-7-*O*-hexoside (μg g^{-1} DW)	caffeic acid (μg g^{-1} DW)	kaempferol-3-*O*-sophoroside-7-*O*-hexoside (μg g^{-1} DW)	quercetin-3-*O*-sophoroside (μg g^{-1} DW)	caffeic acid hexoside isomers (μg g^{-1} DW)	kaempferol-3-*O*-(caffeoyl)-sophoroside-7-O-hexoside (μg g^{-1} DW)	quercetin-3-*O*-(feruloyl)-sophoroside-7-O-hexoside (μg g^{-1} DW)
Genotype (M)							
Komatsuna	18.8 ± 2.2 a	2.9 ± 0.5 ab	5.3 ± 0.8 c	5.4 ± 0.2 c	39.0 ± 2.7 c	15.7 ± 0.9 c	28.1 ± 0.5 c
Mibuna	15.9 ± 1.3 ab	4.1 ± 0.6 ab	23.1 ± 4.0 b	20.1 ± 0.8 a	74.6 ± 6.8 b	116.7 ± 12.9 b	212.9 ± 9.2 c
Mizuna	10.5 ± 0.4 c	4.4 ± 0.7 a	30.9 ± 2.5 a	8.8 ± 1.0 b	121.9 ± 5.6 a	189.6 ± 10.8 a	155.9 ± 14.7 b
Pak choi	12.8 ± 1.6 bc	2.6 ± 0.6 b	5 ± 0.4 c	5.8 ± 0.3 c	38.9 ± 2.0 c	14.6 ± 0.6 c	28.3 ± 3.1 c
p-value	0.000	0.026	0.000	0.000	0.000	0.000	0.000
Growth stage (S)							
S1	14.1 ± 1.7	2.6 ± 0.4 b	12.7 ± 2.8 b	9.6 ± 1.9	64.8 ± 10.3	80 ± 23.0	97.7 ± 21.7
S2	14.8 ± 1.1	4.4 ± 0.3 a	19.4 ± 4.3 a	10.5 ± 1.8	72.4 ± 11.0	88.4 ± 22.8	114.9 ± 27.7
p-value	0.695	<0.001	0.010	0.272	0.147	0.377	0.093
M × S							
Komatsuna × S1	22.9 ± 1.8 a	2.4 ± 0.8	3.8 ± 0.6	5.2 ± 0.3 c	42.4 ± 2.7 c	17.1 ± 1.5 d	29.02 ± 0.3 d
Komatsuna × S2	14.7 ± 2.2 bc	3.5 ± 0.4	6.9 ± 0.5	5.6 ± 0.2 c	35.6 ± 4.4 c	14.3 ± 0.4 d	27.1 ± 0.6 d
Mibuna × S1	13.3 ± 1.2 bc	2.7 ± 0.1	16.4 ± 1.1	20.5 ± 0.6 a	59.8 ± 1.6 c	90.8 ± 7.2 c	200.8 ± 11.7 ab
Mibuna × S2	18.4 ± 0.7 ab	5.4 ± 0.1	29.7 ± 5.8	19.7 ± 1.6 a	89.4 ± 2.8 b	142.7 ± 10.4 b	225.1 ± 11.6 a
Mizuna × S1	9.9 ± 0.6 c	3.8 ± 1.4	25.8 ± 2.6	6.7 ± 0.3 c	120.2 ± 11.9 a	197.3 ± 22.4 a	126.8 ± 11.5 c
Mizuna × S2	11.1 ± 0.4 c	5 ± 0.3	35.9 ± 0.2	11 ± 0.1 b	123.6 ± 3.0 a	182 ± 5.0 ab	184.9 ± 10.3 b
Pak choi × S1	10.4 ± 1.1 c	1.5 ± 0.2	4.7 ± 0.5	6.1 ± 0.3 c	36.6 ± 2.5 c	14.7 ± 0.5 d	34.1 ± 3.3 d
Pak choi × S2	15.1 ± 2.3 bc	3.7 ± 0.5	5.2 ± 0.7	5.6 ± 0.5 c	41.2 ± 2.9 c	14.6 ± 1.2 d	22.5 ± 2.2 d
p-value	<0.001	0.423	0.073	0.005	0.012	0.005	0.002

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$). Microgreens growth stages were compared according to Student's *t*-test.

Table 6. *Cont.*

Source of variance	kaempferol-3-*O*-(coumaroyl) -sophoroside-7-*O*-hexoside (µg g^{-1} DW)	kaempferol-3-*O*-(synapoyl)-sophoroside-7-*O*-hexoside (µg g^{-1} DW)	kaempferol-3-*O*-(feruloyl)-sophoroside-7-*O*-hexoside (µg g^{-1} DW)	coumaroyl quinic acid isomer 1 (µg g^{-1} DW)	naringin (µg g^{-1} DW)	feruloyl quinic acid isomer (µg g^{-1} DW)	apigenin-7-*O*-rutinoside (µg g^{-1} DW)
Genotype (M)							
Komatsuna	5.1 ± 1.6 c	98.8 ± 11.2 b	17.0 ± 2.0 c	15.2 ± 6.7 a	1.3 ± 0.0 c	7.0 ± 1.1 a	2.4 ± 0.3 b
Mibuna	18.5 ± 0.8 b	378.2 ± 27.4 a	96.0 ± 8.8 b	1.7 ± 0.7 b	1.5 ± 0.1 c	5.3 ± 0.3 b	5.0 ± 1.2 a
Mizuna	28.3 ± 1.9 a	412.1 ± 27.8 a	167.9 ± 13.5 a	3.4 ± 1.4 b	3.0 ± 0.1 a	3.1 ± 0.2 c	4.5 ± 0.2 a
Pak choi	1.9 ± 0.1 d	81.4 ± 18.8 b	11.3 ± 2.0 c	14.7 ± 6.2 a	2.6 ± 0.2 b	4.9 ± 1.0 b	5.8 ± 0.2 a
p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Growth stage (S)							
S1	11.2 ± 3.0 b	287.0 ± 50.3 a	61.3 ± 15.6 b	0.5 ± 0.1 b	1.9 ± 0.2 b	4.1 ± 0.5 b	4.7 ± 0.7
S2	15.7 ± 3.5 a	198.3 ± 42.7 b	84.8 ± 23.3 a	17.0 ± 3.8 a	2.3 ± 0.3 a	6.1 ± 0.6 a	4.2 ± 0.4
p-value	<0.001	<0.001	0.015	<0.001	<0.001	0.018	0.504
M × S							
Komatsuna × S1	1.5 ± 0.3 e	120.8 ± 12.3	12.7 ± 0.9 e	0.7 ± 0.1 b	1.2 ± 0.1 c	5.1 ± 1.6 bcd	1.7 ± 0.3 c
Komatsuna × S2	8.6 ± 0.1 d	76.9 ± 1.0	21.2 ± 0.3 e	29.7 ± 3.6 a	1.4 ± 0.1 c	8.8 ± 0.5 a	3.1 ± 0.1 bc
Mibuna × S1	17.2 ± 0.9 c	431 ± 23.8	78.6 ± 2.5 d	0.1 ± 0.1 b	1.3 ± 0.1 c	5.8 ± 0.2 abc	6.9 ± 1.7 a
Mibuna × S2	19.9 ± 0.7 c	325.5 ± 20.3	113.5 ± 8.7 c	3.3 ± 0.1 b	1.6 ± 0.1 c	4.8 ± 0.3 bcd	3.0 ± 0.1 bc
Mizuna × S1	24.4 ± 0.5 b	472.9 ± 13.0	138.3 ± 5.7 b	0.3 ± 0.1 b	2.8 ± 0.1 a	2.7 ± 0.1 cd	4.2 ± 0.2 abc
Mizuna × S2	32.3 ± 1.2 a	351.3 ± 2.6	197.6 ± 2.2 a	6.4 ± 0.5 b	3.2 ± 0.1 a	3.5 ± 0.3 cd	4.9 ± 0.1 ab
Pak choi × S1	1.7 ± 0.1 e	123.3 ± 1.8	15.6 ± 1.0 e	1.0 ± 0.1 b	2.2 ± 0.1 b	2.7 ± 0.2 d	5.8 ± 0.2 ab
Pak choi × S2	2.0 ± 0.1 e	39.5 ± 0.8	6.9 ± 0.7 e	28.4 ± 1.5 a	3.1 ± 0.1 a	7.2 ± 0.1 ab	5.7 ± 0.3 ab
p-value	<0.001	0.059	<0.001	<0.001	0.005	0.002	0.004

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$). Microgreens growth stages were compared according to Student's *t*-test.

Table 6. *Cont.*

Source of variance	ferulic acid (µg g^{-1} DW)	quercetin-3-*O*-glucuronide (µg g^{-1} DW)	luteolin-3-*O*-rutinoside (µg g^{-1} DW)	feruloyl glycoside (µg g^{-1} DW)	Kaempferol-3-*O*-rutinoside (µg g^{-1} DW)	apigenin-7-rhamnoside-4-rutinoside (µg g^{-1} DW)	∑ phenolic acids (µg g^{-1} DW)
Genotype (M)							
Komatsuna	20.5 ± 3.1 d	252.0 ± 20 d	1.6 ± 0.2 c	79.7 ± 5.2 c	2.1 ± 0.5 b	11.9 ± 3.7 b	630 ± 30 d
Mibuna	40.0 ± 2.5 c	319.7 ± 13 c	1.3 ± 0.1 c	113.6 ± 4.8 b	2.6 ± 0.5 b	11.6 ± 2.9 b	1462 ± 45 b
Mizuna	62.1 ± 4.9 a	484.3 ± 21 b	12.1 ± 1.5 a	196.9 ± 20.5 a	8.6 ± 2.2 a	25.0 ± 3.5 a	1933 ± 61 a
Pak choi	48.6 ± 5.0 b	674.8 ± 30 a	2.3 ± 0.2 b	82.8 ± 5.0 c	3.8 ± 0.3 b	10.5 ± 3.3 b	1053 ± 33 c
p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Growth stage (S)							
S1	34.4 ± 4.2 b	389.4 ± 47 b	3.3 ± 1.0 b	99.6 ± 10.1 b	5.3 ± 1.5	7.4 ± 1.8 b	1191 ± 141 b
S2	51.2 ± 5.1 a	475.9 ± 52 a	5.4 ± 1.8 a	136.9 ± 18.8 a	3.3 ± 0.4	22.1 ± 1.9 a	1348 ± 152 a
p-value	<0.001	<0.001	0.024	0.002	0.183	<0.001	<0.001
M × S							
Komatsuna × S1	13.5 ± 0.3 f	208.7 ± 7	1.3 ± 0.1 d	68.5 ± 1.8 f	1.5 ± 0.6 b	3.7 ± 0.1	564 ± 7
Komatsuna × S2	27.5 ± 0.9 e	295.4 ± 6	1.9 ± 0.2 cd	90.8 ± 2.7 de	2.6 ± 0.9 b	20.0 ± 0.3	696 ± 15
Mibuna × S1	34.7 ± 1.2 d	293.3 ± 9	1.1 ± 0.1 d	104.7 ± 4.7 cd	2.6 ± 0.5 b	5.3 ± 1.0	1387 ± 33
Mibuna × S2	45.2 ± 1.3 c	346.1 ± 9	1.5 ± 0.1 d	122.4 ± 3.9 c	2.6 ± 1.0 b	18.0 ± 0.9	1538 ± 59
Mizuna × S1	51.8 ± 3.3 c	440.7 ± 12	8.8 ± 0.4 b	151.8 ± 4.5 b	13.5 ± 0.6 a	17.5 ± 1.2	1820 ± 70
Mizuna × S2	72.4 ± 0.4 a	527.9 ± 11	15.5 ± 0.1 a	242.0 ± 7.3 a	3.6 ± 0.5 b	32.6 ± 1.1	2047 ± 31
Pak choi × S1	37.5 ± 0.5 d	615.1 ± 25	1.9 ± 0.1 cd	73.3 ± 2.7 ef	3.5 ± 0.5 b	3.1 ± 0.1	995 ± 37
Pak choi × S2	59.7 ± 0.8 b	734.4 ± 15	2.7 ± 0.1 c	92.3 ± 5.1 de	4.2 ± 0.2 b	17.9 ± 0.9	1112 ± 27
p-value	0.005	0.155	<0.001	<0.001	<0.001	0.211	0.511

Different letters within each column indicate significant differences according to a Tukey–Kramer HSD test ($p = 0.05$). Microgreens growth stages were compared according to Student's *t*-test.

4. Discussion

4.1. Yield Characteristics

The optimal harvest stage for microgreens should combine high yield and high crop turnover while ensuring prime sensory and functional quality. Most available research on microgreens makes a clear differentiation with sprouts based on the harvesting of microgreens without roots at the substrate-hypocotyl interface. However, the exact definition of microgreens with respect to developmental stage at the time of harvest remains relatively vague. In their review on microgreens, Kyriacou et al. (2016a) defined microgreens as greens harvested upon the appearance of the first pair of true leaves, when cotyledons are fully expanded and still turgid. But the developmental stage at harvest actually varies between different works, with certain researchers harvesting at the cotyledonary stage (Waterland et al., 2017; Klopsch et al., 2019), others at the appearance of the first true leaf (Di Gioia and Santamaria, 2015; De la Fuente et al., 2019; Di Bella et al., 2020) and yet others at the second true leaf (Xiao et al., 2014; Waterland et al., 2017; Kyriacou et al., 2019a, 2020). In terms of fresh and dry yield, the current work demonstrated that lower-yielding genotypes (e.g., Mizuna and Pak Choi) present higher increments of yield increase from the first to the second true-leaf stage compared to the faster-growing and higher-yielding genotypes (e.g., Komatsuna and Mibuna). Delaying harvest of these slower-growing genotypes until the appearance of the second true leaf may allow for significant return in yield (e.g., 80.1% in Mizuna) but this practice presently reduced crop turn-over by 41.7% owing to the increase in crop cycle from 7 to 12 days. Therefore, the decision to harvest at S2 instead of S1 must be analyzed in terms of yield for each genotype, with harvesting at S2 seeming economically sound for low-yielding genotypes.

4.2. Mineral Composition

Minerals are essential components of the human diet for preventing nutritional disorders and facilitating metabolic and homeostatic regulation through their multiple functionalities [36]. Fresh fruits and vegetables contribute approximately 35, 24, 11 and 7% of the human dietary intake in macrominerals K, Mg, P, and Ca, respectively (Levander, 2019). With respect to the mineral composition of microgreens, the present study is largely in agreement

with previous ones that defined K, Ca, P and Mg as the most abundant macrominerals and Mn, Fe and Zn as the most abundant microminerals (Xiao et al., 2016; Di Gioia et al., 2017; Kyriacou et al., 2020). Moreover, several researchers have posited that certain macro- and microminerals (Mg, Ca, Mn, Fe, Zn, Mo and Se) may be found in higher concentrations in microgreens compared to the full developmental stage of the same genotypes (Xiao et al., 2012; Pinto et al., 2015; Waterland et al., 2017). An additional advantage claimed for microgreens is their higher mineral bioavailability owing to the suppression of phytate concentration during germination (Liang et al., 2009; De la Fuente et al., 2019). However, the capacity for uptake and accumulation of minerals varies widely with genotype, as presently demonstrated for genotypes of the Brassicaceae and previously for other genotypes (Przybysz et al., 2016a, 2016b). Although nutrient supplementation and growth conditions may modulate the mineral content of microgreens Renna et al. (2016), the current study highlighted genotype selection as a key factor for designing microgreens of particular mineral profiles; e.g., Pak Choi and Komatsuna yielded microgreens high in P and K, Mibuna yielded Ca-rich and Se-rich microgreens and Mizuna Mo-rich microgreens. From a nutritive aspect, it is also noteworthy that the Na/K ratio, high levels of which are implicated in cardiovascular stress (Choi et al., 2005), varies widely with microgreens genotypes as it was presently found three-fold higher in Mizuna and Pak Choi compared to Komatsuna and Mibuna.

The current study moreover highlighted that developmental changes in the mineral profiles of microgreens differ according to mineral element and genotype. Calcium concentration consistently increased in all species, which might relate to the increasing demands for mechanical support in the developing microgreens. However, P and Na concentrations and that of most microminerals (Mo, Se and Zn) was nonresponsive to the growth stage. Macro-minerals K and Mg varied between growth stages according to genotype, with a prevailing trend for decrease in K and increase in Mg concentration from S1 to S2. Analogous findings were reported by Waterland et al. (2017), who found higher mineral content on a dry weight basis in kale microgreens than in adult kale leaves. They found higher K concentration at the cotyledonary stage than the two-leaf stage but no change in K, P, Mg and Ca and most microminerals between the two-leaf and baby-leaf (four-leaf)

stages. However, on a fresh weight basis, they found maximum mineral content at the baby-leaf (four to six leaves) stage compared to both microgreens and adult leaves.

Pinto et al. (2015), on the other hand, reported mineral concentrations on a fresh weight basis and found lettuce microgreens lower in N, P, K and Ca but higher in Fe, Mn, Zn, Mo and Se than their adult counterparts. Waterland et al. (2017) highlighted that discrepancies in mineral concentrations may derive from variation in water content between genotypes and developmental stages, reporting decrease in water content up to 15% between microgreens and adult leaves. Although lower water content (higher dry matter) was presently also observed between stages S1 and S2, the nominal difference was limited (0.5%) and does not justify the variation observed mainly in macrominerals. Limited variation in water as opposed to mineral content was also reported for lettuce microgreens and adult leaves by Pinto et al. (2015), who reported higher content of most minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo) in microgreens than mature leaves. In the context of the present study, it may be inferred that mineral content on a dry weight basis presents wider differences between genotypes than between growth stages. Variation between growth stages is negligible for most microminerals whereas for Ca, K, Mg, Mn and Fe it depends on genotype and cannot be attributed mainly to increasing dry matter content. The present findings enrich the rather limited reference base for microgreens mineral content.

4.3. Antioxidant Capacity

The antioxidant radical-quenching activity may vary significantly between leafy genotypes, especially between red and green ones, with the antioxidant capacity of the former being generally higher (Nicolle et al., 2004), hence their inclusion in the human diet is expected to counter the impact of oxidative stress. The antioxidant value of colored microgreens has been posited by previous workers (Brazaitytė et al., 2013) and is further corroborated by the present study where the purple microgreens of Komatsuna and Pak Choi demonstrated higher in vitro antioxidant capacity than the green Mizuna and Mibuna. Moreover, the antioxidant value of microgreens seems more variable by genotype than growth stage. Although the antioxidant capacity of certain species (e.g., Komatsuna and Mizuna) may be higher at the first-leaf stage, this phenomenon cannot be generalized since in other genotypes (e.g., Pak Choi and Mibuna) differences in antioxidant capacity from the first to the second-leaf stage tend to be nonsignificant.

Lutein and β-carotene are the key hydrophobic carotenoid molecules found in microgreens that have been shown to exercise lipophilic antioxidant capacity owing to the light-absorbing and ROS-quenching properties of their conjugated double bond-rich polyene chains (Young and Lowe, 2001). Lutein has thus been administered as a nutritional supplement for ophthalmic protection against short wavelength radiation and light-induced macular damage (Kvansakul et al., 2006). Biological activity is also demonstrated by β-carotene that constitutes a precursor of vitamin A, which is essential for immune and ophthalmic functions (Young and Lowe, 2001). Both carotenoid molecules appear more variable across genotypes than between the first and second-true leaf stages of ontogeny, with β-carotene having showed no response to the growth stage whereas lutein concentration demonstrated a genotype-specific increase at the appearance of the second leaf. It is therefore questionable whether delaying harvest to this stage would significantly benefit the bioactive value of microgreens derived from carotenoids. A predominantly genotype-specific variability across growth stages also seems to be the prevailing pattern for the concentration of chlorophyll in microgreens. Similar findings highlighting the significant cultivar-growth stage interaction for chlorophyll and carotenoid pigments were previously demonstrated for red and green butterhead lettuce cultivars harvested at the microgreens and mature growth stages (El-Nakhel et al., 2020). Contrarily, Klopsch et al. (2019) and Heinze et al. (2018) reported a significant increase of chlorophylls and carotenoids from the cotyledonary microgreens to mature-leaf stage. Genotype-growth stage interaction aside, it may be inferred that the brief interval in ontogeny from S1 to S2 may confound variation in these pigments that constitute central components of the developing photosynthetic apparatus biosynthesized according to the growing light-harvesting requirements (Saini and Keum, 2018).

Overall, the ascorbate concentration (73.8 – 119.3 mg kg^{-1} fw) obtained in the four brassicaceous genotypes of the present study is in the low end of the range reported in the available literature on microgreens (Xiao et al., 2015; Ebert et al., 2017; De la Fuente et al., 2019). Although higher ascorbate content is generally expected in microgreens than sprouts, owing to microgreens' longer photosynthetic activity that provides hexose products necessary for ascorbate biosynthesis (Smirnoff and Wheeler, 2000), this difference is not always pronounced at close stages of ontogeny. Developmental variation in microgreens

ascorbic acid concentration exhibits a strongly genotype-dependent effect with nonsignificant change in some genotypes (e.g., Mibuna) as opposed to others. Moreover, variation along developmental stages is not uniform across genotypes, with increase from S1 to S2 being the common response in most genotypes but decrease also being observed in others (e.g., Pak Choi). The current observation of cultivar-growth stage interaction for ascorbate concentration in brassicaceous microgreens corroborates the findings of Di Bella et al. (2020) in their comparative study of sprouts, microgreens and baby leaves of two Brassica oleracea L. cultivars and those of El-Nakhel et al. (2020) on butterhead lettuce microgreens vs. mature leaves.

4.4. Volatile Organic Compounds (VOCs)

The brassicaceous microgreens VOCs profile is dominated by isothiocyanate compounds that constitute catabolic products of glucosinolate metabolism. In the present study, this is suggested by the balanced patterns in the relative abundance of key isothiocyanates observed in different genotypes and growth stages. For instance, 3-butenyl isothiocyanate and allyl isothiocyanate varied inversely between genotypes, the former being found in higher relative abundance at the first-leaf stage and the latter at the second-leaf stage. Changes in the profile and concentration of glucosinolates and their catabolic products have been demonstrated in different Brassicaceae genotypes and stages of ontogeny, with both categories generally found in higher concentrations at the microgreens stage compared to the mature-leaf stage (Heinze et al., 2018). Furthermore, the glucosinolates and their breakdown products have been shown to differ in relative abundance and complexity with progressive ontogeny of brassicaceous genotypes, with more abundant and diverse products formed in Pak Choi microgreens than kale microgreens (Klopsch et al., 2019). The relative abundance of the major VOCs presently identified varied between genotypes more extensively than between growth stages. Analogous variation in the total isothiocyanates and their bioaccessible fraction was demonstrated for different brassicaceous microgreens by De la Fuente et al. (2019). Moreover, response to the growth stage was not uniform across genotypes. It is worth noting however that the dominant volatile aromatic component in the microgreens volatile profile (3-butenyl isothiocyanate) had higher relative abundance at S1 than S2, whereas the reverse trend was dominant for the rest of the identified VOCs. This suggests that 3-butenyl isothiocyanate fueled the production of glucosinolate catabolites with an enhanced presence

at S2 and corroborates previous studies reporting higher content of glucosinolate catabolites at earlier stages of ontogeny (Klopsch et al., 2019).

Another noteworthy change observed at the second-leaf stage is that the cyclic monoterpene limonene, which imparts a characteristic citrus-like aroma, increased in all genotypes during transition from S1 to S2. Similar increase at S2 was also observed for the aldehydes nonanal, which imparts a sweet floral (orange-flower-like) scent, and for trans-2-hexenal, which imparts a fresh leafy green scent (The Good Scents Company, 2021). Therefore, the development of more distinctive aroma profiles during transition from S1 to S2 in brassicaceous microgreens may be inferred. However, these changes cannot be termed universal since the volatile profile of certain genotypes seems less prone to developmental change, with Mizuna microgreens as a current example.

4.5. Anthocyanins

Microgreens are a specialty crop that has become a popular gastronomic ingredient owing, among others, to their vivid colors (Kyriacou et al., 2016a). Anthocyanins are responsible for the purple, blue and red pigmentation of microgreens. However, their role in plants extends to biological functions such as ROS scavenging, protection from radiation and other stress conditions as well as to defense against pathogens (Chaves-Silva et al., 2018; Di Gioia et al., 2020). The bioactive value of anthocyanins for human health has also been posited, hence the consumption of colored greens and fruits is encouraged (He and Monica Giusti, 2010). The anthocyanin concentrations in the green genotypes Mibuna and Mizuna presently studied were shown not to change in response to the growth stage. However, developmental changes in the red genotypes were inconsistent as anthocyanin concentration increased in Komatsuna during transition to S2 as opposed to Pak Choi where maximal concentrations were observed at S1. Moreover, only certain anthocyanin molecules demonstrated significant positive correlation with the hydrophilic antioxidant capacity of microgreens extracts, whereas other molecules and the total anthocyanins concentration did not (data not shown). This is not surprising as the in vitro antioxidant capacity of naturally occurring anthocyanins in the juice or aqueous extracts of pigmented fruits, vegetables and flowers is not consistent across genotypes; moreover, although it has been documented in vitro for several genotypes, analogous in vivo activity has not been widely demonstrated with respect to human consumption (Granato et al., 2018). It is therefore apparent that apart

from the clear divide in the relative abundance of anthocyanins between green- and purple-colored microgreens, variation with developmental stage is largely genotype-specific and the choice of harvest stage based on visual quality must be defined on a genotype-by-genotype case.

4.6. Polyphenols

Flavonol glycosides, hydroxycinnamic acids and their derivatives were the dominant classes of phenols present in the four brassicaceous microgreens examined. The current findings are largely consistent with previous studies on brassicaceous microgreens that identified glycosides of quercetin and kaempferol as the main flavonoid glycosides present (Klopsch et al., 2019), and the derivatives of caffeic and ferulic acids among the main hydroxycinnamic acids found in conjugation with sugars or other hydroxycinnamic acid moieties (Harbaum et al., 2007; Olsen et al., 2009; Sun et al., 2013). Despite broad qualitative similarities in their phenolic composition, quantitative differences between genotypes were wide, confirming analogous findings on other brassicaceous microgreens (Waterland et al., 2017). It is further apparent that the natural pigmentation of microgreens is not a sound indication of their phenolic content. Although anthocyanins comprise a class of high-molecular-weight glycosylated polyphenols, their abundance in pigmented microgreens does not constitute a criterion for their content in total polyphenols, as presently demonstrated by the higher phenolic content of the green microgreens Mibuna and Mizuna compared to the purple microgreens Komatsuna and Pak Choi. The current study further highlighted that genotype is potentially a far more significant source of variation in phenolic content than growth stage, especially in the narrow window of ontogeny presently examined.

The phenolic composition at the cotyledonary (rootless sprouts), microgreen (first true leaf) and baby-leaf (third true leaf) stages of three Brassica genotypes was examined by Di Bella et al. (2020) who identified a general tendency for declining phenolic content, with the content being more often higher in sprouts vs. microgreens than in microgreens vs. baby leaves. However, similarly to the current findings, this trend was underlined by genotype × growth stage interaction rendering the effect of ontogeny at the early post-germination stages largely genotype-dependent. Analogous genotype × growth stage interaction was reported for different amaranth genotypes harvested at the sprout and microgreen stages (Ebert et al., 2017). It is known that the germination process drives major events in the biosynthesis and

metabolism of polyphenols through the phenylpropanoid pathway that may generate differences between successive stages of ontogeny (Dueñas et al., 2009). The absence of significant growth stage effect observed on several of the 20 phenolic components presently identified is consistent with the findings of Di Bella et al. (2020) that post-germination differences in phenolic composition between first-leaf and third-leaf microgreens (or baby leaves) tend to be minimal although both stages are characterized by higher phenolic content than the mature leaves of the corresponding genotypes (Klopsch et al., 2019; El-Nakhel et al., 2020).

5. Conclusions

The ontogenetic stages for harvesting microgreens range from the cotyledonary stage to the emergence of the second true leaf. In the current study, the mineral content of brassicaceous microgreens presented wider differences between genotypes than growth stages S1 and S2. Variation in most microminerals with growth stage was negligible whereas for Ca, K, Mg, Mn and Fe it was genotype-dependent. Antioxidant capacity was in certain genotypes higher at S1 but growth stage differences were nonsignificant in other genotypes. Key carotenoids lutein and β-carotene varied widely by genotype whereas β-carotene showed no response to the growth stage and lutein a genotype-dependent increase at S2. It is therefore questionable whether delaying harvest to S2 would significantly benefit the bioactive value of microgreens derived from carotenoids.

Ascorbate concentration also exhibited genotype-dependent variation with growth stage, with nonsignificant change found in certain genotypes (e.g., Mibuna). The volatile components of brassicaceous microgreens comprised mostly isothiocyanate catabolites of glucosinolate metabolism, the relative abundance of which varied mostly by genotype, with certain genotypes (e.g., Mizuna) seeming less prone to developmental changes. The current study further highlighted that against the narrow window of ontogeny examined, genotype is the principal source of variation for microgreens' phenolic content. The absence of significant growth stage effect on many of the phenolic components identified is consistent with previous findings that post-germination differences in phenolic composition between S1 microgreens and baby leaves are minimal. Finally, lower-yielding genotypes register higher relative increase in fresh yield between S1 and S2, compared to faster-growing and

higher-yielding genotypes. Although the optimal harvest stage must be determined for each genotype considering yield increase against crop turnover reduction, harvesting at S2 seems advisable for the lower-yielding genotypes.

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Chapter 6: Preharvest Nutrient Deprivation Reconfigures Nitrate, Mineral and Phytochemical Content of Microgreens

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Preharvest Nutrient Deprivation Reconfigures Nitrate, Mineral, and Phytochemical Content of Microgreens

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Preharvest Nutrient Deprivation Reconfigures Nitrate, Mineral and Phytochemical Content of Microgreens

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Abstract

While imparting gastronomic novelty and sensory delight, microgreens also constitute rudimentary leafy greens packed with nutrients and phytochemicals. As such, they comprise an upcoming class of functional foods. However, apart from bioactive secondary metabolites, microgreens also accumulate antinutritive agents such as nitrate, especially under conducive protected cultivation conditions. The current work examined nutrient deprivation before harvest (DBH), applied by replacing nutrient solution with osmotic water for six and twelve days, as a strategy for reducing microgreen nitrate levels in different species (lettuce, mustard and rocket). The three species were sown on a peat-based substrate, cultivated in a controlled climate chamber, and harvested 18 days after sowing, when the first two true leaves emerged. DBH impact on major constituents of the secondary metabolome, mineral content, colorimetric and yield traits was appraised. Nitrate and mineral content were determined through ion chromatography, phenolic composition through UHPLC-Q-Orbitrap HRMS and carotenoid composition through HPLC-DAD. Nutrient deprivation was effective in reducing nitrate content, however effective treatment duration differed between species and decline was more precipitous in nitrate hyperaccumulating species such as rocket. Quercetin and kaempferol glycosides were the flavonol glycosides most abundant in brassicaceous microgreens, whereas lettuce microgreens were steeped in caffeoyl quinic acid. DBH interacted with species as it increased the total phenolic content of lettuce, decreased that of rocket but did not affect mustard. Further research to link changes in phenolic composition to the sensory and in vivo bioactive profile of microgreens is warranted. Notably, brief (≤ 6 days) DBH can be applied

across species with moderate or no impact on the phenolic, carotenoid and mineral composition of microgreens. Brief DBH applications also have limited impact on microgreens' yield and colorimetric traits hence on the commercial value of the product. They can therefore be applied for reducing microgreen nitrate levels without significantly impacting key secondary metabolic constituents and their potential bioactive role.

Keywords: *antinutritive agents; carotenoids; bioactive value; flavonol glycosides; functional foods; hydroxycinnamic acids; polyphenols*

1. Introduction

Microgreens upgrade the color and sensory palette of modern foods as reflected in the appealing adjectives they have been ascribed, which range from "vegetable confetti" (Kyriacou et al., 2016a, 2019e; Caracciolo et al., 2020; Verlinden, 2020), to "lingerie of the culinary world" (Verlinden, 2020), "functional foods" and "super foods" (Kyriacou et al., 2016a; Palmitessa et al., 2020) and much more. Undoubtedly, microgreens infuse human diets with gastronomic novelty (Verlinden, 2020); moreover, they serve as dietary carriers of health-promoting plant secondary metabolites, while they are concomitantly renowned for their offbeat tastes, alluring colors and subtle textures (Caracciolo et al., 2020; Verlinden, 2020; El-Nakhel et al., 2021; Teng et al., 2021). Microgreens have been commonly used for garnishing fancy dishes, salads and sandwiches (Caracciolo et al., 2020; Paradiso et al., 2020; Teng et al., 2021), but lately they constitute basic ingredients of savory and sweet dishes with unconventional organoleptic profiles (Palmitessa et al., 2020; Paradiso et al., 2020). Noteworthy characteristics of microgreens include compact form, accelerated production, limited space requirement for growth and high crop turnover, all of which render them an appealing specialty product in the modern horticultural supply chain and a crop well-adapted to protected cultivation even in urban environments (Verlinden, 2020; Teng et al., 2021). As stated by El-Nakhel et al. (El-Nakhel et al., 2019), protected cultivation such as indoor growing modules, can produce fresh vegetables of constant quality owing to precise control of growth conditions yearlong.

Plant foods, including microgreen vegetables, are naturally biofortified with an array of bioactive compounds such as vitamins, minerals, and secondary metabolites (Bian et al., 2020; El-Nakhel et al., 2021). Aside from desirable secondary metabolites however,

vegetables can also accumulate antinutritive agents such as nitrate, especially under conducive protected cultivation conditions (Simanavicˇius and Virsˇile, 2018; Bian et al., 2020). The metabolic products of nitrate have come under investigation for possible association with certain types of cancer and the methemoglobinemia syndrome (EuropeanFood Safety Agency (EFSA)., 2008). Excessive accumulation of nitrate is frequently observed in leafy greens and poses a potential peril to human well-being (Cavaiuolo and Ferrante, 2014; Bian et al., 2020), hence tolerance levels for nitrate have been set (Regulation No 1882/2006) by the European Commission for particular species under protected and open-field cultivation (European Commission Regulation (EC) No 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrates in certain foodstuffs., 2006). A cutback on the nitrate content of vegetable foods is therefore desirable in order to underscore their nutritive and nutraceutical value (Anjana et al., 2007).

Nitrate uptake, assimilation, translocation, and accumulation in plants are driven by a manifold of internal (genetic variability, concerted gene expression and enzymatic activity, ontogenetic stages) and external factors (N form, concentration and application time; light intensity, quality and photoperiod; air temperature; CO_2 concentration) that also influence the sensory and phytochemical traits of fresh vegetables (Colla et al., 2018; Kyriacou et al., 2019a; Bian et al., 2020). Nitrate is the main form of nitrogen readily taken up by crops and is vital for protein and nucleic acid biosynthesis (Cavaiuolo and Ferrante, 2014), but also vital for sustaining maximal yields (Colla et al., 2018). However, excessive nitrate fertilization promotes nitrate accumulation in plant tissues (Cavaiuolo and Ferrante, 2014; Colla et al., 2018), especially in petioles, leaves and stems (Anjana et al., 2007; Colla et al., 2018). As stated by Colla et al. (2018), variable preharvest approaches can be considered for lowering nitrate content in plants: (i) diminishing nitrate concentration in the fertigation solution, (ii) replacing nitrate with urea or ammonium, (iii) replacing the nutrient solution with water or a nitrate-free solution for a time period before harvest, (iv) replacing calcium nitrate with calcium chloride, (v) manipulating light spectral composition and intensity and (vi) using low-accumulating genotypes.

Conventionally, microgreens have been regarded as low accumulators of nitrate among salad crops; however, relatively high concentrations of nitrate were detected in some

microgreen species (Lenzi et al., 2019; Teng et al., 2021). Moreover, one of the most popular families exploited for microgreens production is the *Brassicaceae*, which comprises nitrate hyperaccumulators such as rocket (Kyriacou et al., 2016a; Colla et al., 2018). Few studies have dealt with fertigation strategies aimed to reduce nitrate accumulation in microgreens, of which we mention El-Nakhel et al. (El-Nakhel et al., 2021) and Pannico et al. (Pannico et al., 2020) who adopted drastic strategies of fertigation with a ¼ strength nutrient solution (400 μS cm^{-1}) or distilled water for the entire growth cycle, and Palmitessa et al. (Palmitessa et al., 2020) who used ½, ¼ and ⅛ strength and different NH_4:NO_3 molar ratios. Nutrient deprivation strategies for a few days before harvesting, are known for eliciting changes in the plant secondary metabolome (El-Nakhel et al., 2019). Similarly, the potential for eliciting the buildup of bioactive compounds by changing the associated enzymatic activity and gene expression was demonstrated in sprouts under appropriate conducive conditions (Liu et al., 2019). Among numerous elicitors currently investigated on sprouts and microgreens, the most common are abiotic elicitors, which include seed priming with salts, mineral biofortification, and elicitation on mother plants transferred epigenetically to the sprouting seeds (Galieni et al., 2020). Biotic elicitors are another class that have attracted research interest, and these include fungal and yeast inoculation, and the inclusion of bacteria in substrates or seeds. Biostimulation is yet another upcoming area of interest that includes both biotic and abiotic factors as well as their combinatorial effects as elicitors. The most notable changes elicited relate to the products of the phenylpropanoid pathway. Previous studies have highlighted the antihypertensive (Onakpoya et al., 2015) and anti-inflammatory effects (Tajik et al., 2017) of plant-derived phenolics, the composition and concentration of which in vegetables is modulated by genetic, ontogenetic, environmental and cultural factors (Whitaker and Stommel, 2003). Additionally, qualitative differences in phenolic composition were found critical in respect to lipid peroxidation and A549 cell proliferation (Santos et al., 2020). Quercetin derivatives for instance were shown to exert pronounced antioxidant and anti-inflammatory activity and a protective role on other dietary antioxidants present in the human plasma (Terao et al., 2001; Lesjak et al., 2018).

Based on the above, it may be inferred that nutrient deprivation treatments before harvesting microgreens may potentially exert a dual effect: a) reduce the concentration of nitrate, and b) modulate the composition and concentration of secondary metabolites that

contribute to the bioactive value of microgreens. In order to examine these hypotheses, the current study appraised the response of three common microgreen species (lettuce, rocket and mustard), grown in a strictly controlled climate chamber environment, to nutrient deprivation treatments of variable duration applied before harvest. Treatment effects were appraised in terms of yield traits, nitrate and mineral content determined through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), phenolic composition determined through UHPLC-Q-Orbitrap HRMS and carotenoid composition determined through HPLC-DAD. The current study advances our understanding of the utility of nutrient deprivation strategies as a tool for modulating the qualitative attributes of microgreens and reducing their content in anti-nutrients like nitrate.

2. Materials and Methods

2.1. Genetic Material, Climate Chamber Set-Up and Nutrient Solution Treatments

Three nitrate accumulating species from two different families (Brassicaceae and Asteraceae) were grown and harvested at the appearance of the second true leaf: mustard (*Brassica juncea* (L.) Czern cv. Osaka purple; Condor Seed Production, Yuma Arizona, USA), rocket (*Diplotaxis tenuifolia* cv. Wild Rocket, Napoli; CN Seeds Ltd., Pymoor, Ely, Cambrigeshire, UK) and lettuce (*Lactuca sativa* L. cv. Grand Rapids TBR; West Coast Seeds, Delta, British Columbia, Canada). The three species were sown respectively at densities of 60000, 160000 and 50000 seeds m^{-2}, and harvested 18 days after sowing (DAS), when the first two true leaves emerged. All microgreens were cultivated in a controlled climate chamber (MIR-554 growth chamber, Panasonic, Gunma, Japan) at the Department of Vegetable Crops of the Agricultural Research Institute (ARI), Nicosia, Cyprus. Plastic trays (19 × 14 × 6 cm) were filled with a peat-based substrate (pH 5.48 and EC 282 μS cm^{-1}; Special Mixture, Floragard Vertriebs-GmbH, Oldenburg, Germany) mixed with vermiculite (1:1 volume ratio) characterized by the following elements: NO_3 11 mg kg^{-1}, PO_4 140 mg kg^{-1}, K 796 mg kg^{-1}, Ca 2402 mg kg^{-1}, Mg 303 mg k^{-1}, SO_4 235 mg kg^{-1}, Na 540 mg kg^{-1}, expressed on a dry weight basis. Microgreens were subjected to three different fertigation strategies that consisted of nutrient solution termination at different days before harvest (DBH): 0, 6 and 12, to be replaced with osmotic water (pH = 6 ± 0.2 and EC = 150 ± 50 μS cm^{-1}). The adopted nutrient solution was a quarter-strength modified Hoagland

solution (pH = 6 ± 0.2 and EC = 500 ± 50 μS cm^{-1}), described in detail in Kyriacou et al. (Kyriacou et al., 2019b).

The climate chamber was set at 24/18 ± 2 °C, day/night temperatures, corresponding to a photoperiod of 12 h, and a relative humidity in the range of 65-75 ± 5 %. The artificial light of the climate chamber was provided by an LED panel (K5 Series XL 750, Kind LED, California, USA), of which the channels were set at: 45% Red, 10% Green-Yellow, 45% Blue, offering a mean irradiance of 300 ± 15 μmol m^{-2} s^{-1} at canopy level, and wavelengths ranging between 400 and 700 nm while procuring an optimal absorption spectrum for photosynthesis. The trays were rotated during the growth cycle on a daily basis, to secure a homogenous repartition of light, humidity and temperature over the different treatments across the climate chamber.

2.2. Colorimetric Measurement of Microgreens Canopy, Sampling and Yield Assessment

Eighteen DAS, at the two-true-leaf stage, the CIELAB color space parameters (L*, a* and b*) were measured at the microgreens canopy level through a portable Minolta Chroma meter (CR-400, Minolta Co. Ltd., Osaka, Japan), then the hue angle (h◦) and chroma (C*) were calculated. Each tray/replicate received eight measurements across its entire surface. Immediately after, all microgreens were harvested contemporarily by means of scissors by cutting the entire seedlings at substrate level, fresh weight was immediately assessed and expressed as kg fresh weight m^{-2}. A sub-sample of each replicate was dried at 65 °C in a forced-air oven until reaching constant dry weight, which was used to calculate the dry matter percentage. This oven-dried subsample was ground and used for macro-minerals analysis; whereas the remaining part of each sample was stored at -80 °C and lyophilized prior to phytochemical analyses.

2.3. Analysis of Macro- and Micro-minerals by ICP-OES

Microwave-assisted digestion (MLS-1200, Microwave Laboratory Systems, Milestone, Shelton, CT, USA) of about 1 g of desiccated microgreens in 10 mL of 65% HNO3 and 37% HCl (3:1, v/v) was performed and the digested solutions were then brought to 50 mL with ultrapure water (Milli-Q, Merck Millipore, Darmstadt, Germany). Macro-minerals P, K, Ca, Mg and S were determined using ICP-OES spectrometry (Spectroblue, Spectro Ametek,

Berwyn, PA, USA), as detailed in Volpe et al. (Volpe et al., 2015). Calibration curves were established in the range of standard concentrations from 1.0 to 100 μg L^{-1} and expressed in μg g^{-1} dw. Concurrent analysis of standard reference material (BCR CRM 142R - Commission of the European Communities, 1994) enabled accuracy monitoring and recoveries ranged from 86 to 98%.

2.4. Analysis of Carotenoids by HPLC-DAD

Carotenoids were extracted from lyophilized microgreen samples, separated and quantified as previously described by Kyriacou et al. (Kyriacou et al., 2019b) on an Agilent HPLC system (Agilent Technologies, Santa Clara, CA, USA) furnished with a 1200 Series quaternary pump and a 1260 Diode Array Detector Separation. Separation was accomplished with a Gemini C18 (Phenomenex, Torrance, CA, USA) reverse phase column (250 mm × 4.6 mm, 5 μm). Quantification using β-carotene and lutein commercial standards was performed on calibration curves established at six concentrations from 5 to 100 μg ml^{-1} and expressed in mg kg^{-1} DW

2.5. Analysis of Polyphenols by UHPLC-Q-Orbitrap HRMS

Polyphenols were extracted from lyophilized micro-greens with methanol/water (60:40, v/v) and analyzed according to the method detailed in Kyriacou et al. (Kyriacou et al., 2019a). Analysis was performed using a UHPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific, Ma, USA) coupled to a Q-Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fischer Sci-entific, Ma, USA). Polyphenols were separated using a Luna Omega PS 1.6 μm (50 mm × 2.1 mm, Phenomenex) column. Identification and quantification of compounds was accomplished using a Q-Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fischer Scientific, Waltham, MA, USA) operated in fast negative/positive ion switching mode (Thermo Scientific, Bremen, Germany). Data analysis and processing were performed using software Xcalibur v. 3.0.63 (Xcalibur, Thermo Fisher Scientific, Waltham, MA, USA). Phenolic compounds were identified and quantified against calibration curves of available standards and expressed in μg g^{-1} DW. For standards that were not available, identification MS/MS experiments were employed.

2.6. Experimental Design and Statistical Analysis

A completely randomized experimental design was applied. Treatments were replicated three times with each replicate corresponding to a single tray of microgreens. Two-way analysis of variance (ANOVA) with species and nutrient deprivation as the main factors was performed using JMP statistical package (SAS Institute, Inc., Cary, NC, USA). Means were compared and separated according to the Tukey-Kramer HSD test.

3. Results

3.1. Yield Characteristics

Fresh yield was significantly affected by both species and DBH treatment but variability was greater in response to species than it was between different DBH treatments (**Table 1**). The fresh yield of the highest yielding species (mustard) was 85.9% higher than that of the lowest-yielding species (rocket). Fresh yield demonstrated a near linear incremental decrease in response to DBH treatment duration. Increasing DBH from zero to six or from six to 12 days resulted in 5.6% and 11.8% decrease in fresh yield, respectively. Yield in terms of dry weight was significantly affected only by species, however a limited response to DBH was observed for rocket, the dry weight of which increased after 6-DBH and 12-DBH treatments. Dry matter content differed significantly between species with rocket demonstrating overall the highest DM and lettuce the lowest. Rocket was also the species most responsive to DBH with DM increasing with each DBH increment, whereas mustard incurred an increase in DM only at 12 DBH and lettuce no significant change in response to DBH.

Table 1. Yield attributes of three microgreen species (lettuce, mustard, rocket) in response to nutrient deprivation treatment (DBH) applied for 6 or 12 days before harvest. All data are expressed as mean ± standard error, n = 3.

	Fresh weight ($kg\ m^{-2}$)		**Dry weight ($g\ m^{-2}$)**		**DM (%)**	
Species	***		***		***	
DBH	***		n.s.		***	
Species*DBH	n.s.		***		***	
Lettuce	2.35 ± 0.06	b	106.25 ± 1.39	b	4.53 ± 0.08	c
Mustard	2.68 ± 0.07	a	162.18 ± 1.38	a	6.08 ± 0.13	b
Rocket	1.44 ± 0.05	c	100.71 ± 2.27	c	7.09 ± 0.4	a
0	2.33 ± 0.2	a	122.28 ± 11.11		5.31 ± 0.27	c
6	2.2 ± 0.18	b	124.03 ± 9.67		5.76 ± 0.34	b
12	1.94 ± 0.18	c	122.83 ± 9		6.63 ± 0.55	a
Lettuce,0	2.53 ± 0.04		108.74 ± 0.91	b	4.3 ± 0.04	d
Lettuce,6	2.38 ± 0.01		107.4 ± 1.29	b	4.51 ± 0.04	d
Lettuce,12	2.14 ± 0.06		102.6 ± 3.23	b	4.79 ± 0.07	d
Mustard,0	2.89 ± 0.03		165.62 ± 2.04	a	5.73 ± 0.12	c
Mustard,6	2.72 ± 0.03		162.52 ± 1.2	a	5.98 ± 0.02	c
Mustard,12	2.43 ± 0.03		158.4 ± 1.99	a	6.52 ± 0.11	b
Rocket,0	1.57 ± 0.05		92.47 ± 0.36	c	5.91 ± 0.21	c
Rocket,6	1.5 ± 0.02		102.17 ± 1.38	b	6.81 ± 0.01	b
Rocket,12	1.26 ± 0.03		107.49 ± 1.42	b	8.57 ± 0.11	a

ns, *** Nonsignificant or significant at $p \leq 0.001$, respectively. Different letters within each column indicate significant differences according to Tukey-Kramer HSD test ($p = 0.05$).

3.2. Canopy Colorimetry

Genotype had a significant effect on all colorimetric attributes of the microgreens canopy determined in the CIELAB color space (**Table 2**). Nutrient deprivation treatments however did not have a horizontal effect on all colorimetric attributes. DBH treatments affected only the variables defining color intensity (a*, b* and chroma-C*) whereas their effect on the lightness (L*) and the quality of microgreens color denoted by hue angle (h°) was non-significant, with the exception of mustard that incurred a significant reduction in L* after 12-DBH treatment. Lettuce microgreens had the least dark canopy and mustard the darkest.

Mustard microgreens had the lowest intensity of green and yellow color, expressed as the lowest negative values of color component a* and the lowest positive values of color component b*. However, the lowest overall color intensity (chroma) was demonstrated by rocket and the highest by lettuce. With respect to the DBH treatments, the most intensely green colored canopy was obtained from the control (zero DBH), which however did not differ significantly from the 6-DBH treatment. The 6-DBH and 12-DBH treatments also did not differ significantly, despite a trend in nominal values for declining greenness as the DBH increased from six to twelve days. The overall color intensity of microgreens (Chroma) did not change with nutrient deprivation for mustard and rocket but decreased significantly in lettuce when DBH was extended to 12 days.

Table 2. Canopy colorimetric attributes of three microgreen species (lettuce, mustard, rocket) in response to nutrient deprivation treatment (DBH) applied for 6 or 12 days before harvest. All data are expressed as mean ± standard error, n = 3.

	L* **(0-100)**		**a*** **(-60/+60)**		**b*** **(-60/+60)**		**Chroma** $\sqrt{(a^2 + b^2)}$		**HUE** **(0-360)°**	
Species	***		***		***		***		***	
DBH	n.s.		**		**		**		n.s.	
Species*DBH	**		n.s.		n.s.		*		n.s.	
Lettuce	45.29 ± 1.76	a	-8.59 ± 0.24	b	28.73 ± 0.74	a	29.99 ± 0.78	a	106.65 ± 0.18	b
Mustard	31.88 ± 1.15	c	-6.15 ± 0.26	a	21.96 ± 0.42	c	22.81 ± 0.46	b	105.6 ± 0.46	b
Rocket	37.9 ± 0.63	b	-8.42 ± 0.1	b	24.46 ± 0.36	b	13.5 ± 0.15	c	109.03 ± 0.29	a
0	38.47 ± 3.18		-8.15 ± 0.47	b	26.02 ± 1.3	a	23.16 ± 2.73	a	107.42 ± 0.73	
6	39.17 ± 1.91		-7.78 ± 0.37	ab	25.19 ± 0.98	ab	22.24 ± 2.4	a	107.16 ± 0.54	
12	37.44 ± 1.47		-7.23 ± 0.43	a	23.95 ± 0.94	b	20.9 ± 2.1	b	106.7 ± 0.5	
Lettuce,0	50.17 ± 0.39	a	-9.29 ± 0.07		30.91 ± 0.21		32.28 ± 0.22	a	106.73 ± 0.1	
Lettuce,6	45.2 ± 2.09	ab	-8.52 ± 0.31		28.78 ± 0.24		30.01 ± 0.28	ab	106.48 ± 0.52	
Lettuce,12	40.5 ± 3.05	bc	-7.96 ± 0.37		26.51 ± 1.3		27.68 ± 1.34	b	106.73 ± 0.32	
Mustard,0	28.66 ± 0.7	d	-6.5 ± 0.72		22.82 ± 0.58		23.74 ± 0.72	c	105.84 ± 1.4	
Mustard,6	33.6 ± 2.33	cd	-6.35 ± 0.1		22.29 ± 0.64		23.17 ± 0.63	c	105.91 ± 0.38	
Mustard,12	33.39 ± 1.44	cd	-5.59 ± 0.28		20.78 ± 0.49		21.52 ± 0.53	c	105.05 ± 0.47	
Rocket,0	36.56 ± 1.52	cd	-8.67 ± 0.13		24.32 ± 1.16		13.47 ± 0.47	d	109.68 ± 0.58	
Rocket,6	38.71 ± 0.32	bc	-8.48 ± 0.17		24.52 ± 0.3		13.53 ± 0.11	d	109.1 ± 0.46	
Rocket,12	38.44 ± 1.01	bc	-8.12 ± 0.06		24.55 ± 0.38		13.49 ± 0.16	d	108.32 ± 0.16	

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey-Kramer HSD test ($p = 0.05$).

3.3. Nitrate and Mineral Content

The nitrate content of microgreens was significantly affected by both genotype and DBH treatment, moreover a significant genotype-DBH interaction was observed (**Table 3**). Mean nitrate content in rocket microgreens (1111±342 μg g^{-1}) was near three-fold that of lettuce and mustard. Significant reduction in nitrate levels was observed with the DBH treatments. Across genotypes, mean nitrate content was reduced to 36.3% and 13.5% of the control after six and twelve days of DBH treatment, respectively. However, a variable response to DBH treatments was observed in the three studied genotypes with rocket demonstrating the highest proportional reduction in nitrate content at both the six (-72.9%) and twelve-day treatments (-90.3%). Lettuce microgreens on the other hand incurred a significant decrease in nitrate levels only after twelve days of DBH whereas mustard microgreens incurred a significant reduction after six days of DBH but no further reduction at twelve days. Phosphorus and potassium content varied with genotype significantly with highest P and lowest K found in mustard, as opposed to lettuce microgreens that combined the lowest levels of P and the highest levels of K. Both P and K declined with DBH treatment, although significant reduction in P was observed after six and twelve days of DBH whereas significant K reduction was demonstrated only after twelve days of DBH. Reduction in P content after six days of DBH was however practically limited as it accounted for only 14.6% of the control levels (zero DBH). Divalent cations Ca and Mg varied in concentration significantly between genotypes but their response to DBH treatments was non-significant. Finally, S content was overall highest in mustard and lowest in lettuce microgreens and declined with increasing DBH treatment duration. A significant genotype-DBH interaction was observed as lettuce incurred significant reduction in S content after 12 days of DBH treatment as opposed to the microgreens of the brassicaceous species mustard and rocket that incurred significant reduction after six days of DBH.

Table 3. Nitrate and mineral content of three microgreen species (lettuce, mustard, rocket) in response to nutrient deprivation treatment (DBH) applied for 6 or 12 days before harvest. All data are expressed as mean ± standard error, n = 3.

Source of variance	Nitrate (mg kg^{-1} FW)		P (mg g^{-1} DW)		K (mg g^{-1} DW)		Ca (mg g^{-1} DW)		Mg (mg g^{-1} DW)		S (mg g^{-1} DW)	
Species	***		***		***		***		***		***	
DBH	***		***		***		n.s.		n.s.		***	
Species*DBH	***		n.s.		n.s.		*		*		***	
Lettuce	313.36 ± 53.52	b	3.55 ± 0.13	c	97.68 ± 1.11	a	14.66 ± 0.39	c	7.75 ± 0.13	b	3.01 ± 0.16	c
Mustard	386.81 ± 82.58	b	4.97 ± 0.14	a	69.57 ± 1.47	c	21.32 ± 0.46	a	9.90 ± 0.20	a	4.39 ± 0.37	a
Rocket	1111.22 ± 341.57	a	4.34 ± 0.21	b	77.78 ± 1.11	b	19.26 ± 0.33	b	7.91 ± 0.18	b	4.08 ± 0.45	b
0	1208.73 ± 313.05	a	4.86 ± 0.23	a	84.35 ± 4.34	a	18.48 ± 0.85		8.35 ± 0.28		4.99 ± 0.38	a
6	439.25 ± 56.77	b	4.15 ± 0.19	b	82.2 ± 4.21	a	18.42 ± 1.00		8.43 ± 0.38		3.66 ± 0.17	b
12	163.40 ± 19.30	c	3.86 ± 0.22	c	78.47 ± 4.26	b	18.34 ± 1.28		8.79 ± 0.46		2.84 ± 0.14	c
Lettuce,0	502.98 ± 9.23	bcd	3.99 ± 0.06		101.24 ± 1.54		15.22 ± 0.48	d	7.99 ± 0.02	c	3.48 ± 0.05	bcd
Lettuce,6	300.83 ± 17.81	de	3.54 ± 0.06		97.41 ± 0.69		14.69 ± 0.40	d	7.76 ± 0.32	c	3.13 ± 0.06	cde
Lettuce,12	136.27 ± 15.18	e	3.12 ± 0.09		94.39 ± 0.5		14.07 ± 1.03	d	7.52 ± 0.19	c	2.42 ± 0.04	e
Mustard,0	686.13 ± 18.91	b	5.45 ± 0.14		74.19 ± 1.17		20.15 ± 0.63	abc	9.38 ± 0.29	ab	5.72 ± 0.09	a
Mustard,6	356.09 ± 8.58	cde	4.84 ± 0.15		68.7 ± 1.97		21.21 ± 0.65	ab	9.84 ± 0.18	a	4.17 ± 0.25	b
Mustard,12	118.2 ± 7.44	e	4.63 ± 0.08		65.81 ± 1.64		22.59 ± 0.41	a	10.49 ± 0.21	a	3.28 ± 0.16	cd
Rocket,0	2437.09 ± 188.52	a	5.14 ± 0.18		77.63 ± 2.29		20.06 ± 0.37	abc	7.68 ± 0.18	c	5.76 ± 0.22	a
Rocket,6	660.84 ± 26.44	bc	4.07 ± 0.05		80.5 ± 0.67		19.36 ± 0.30	bc	7.69 ± 0.34	c	3.67 ± 0.15	bc
Rocket,12	235.74 ± 13.41	de	3.82 ± 0.12		75.21 ± 1.49		18.35 ± 0.55	c	8.35 ± 0.27	bc	2.81 ± 0.14	de

ns,*, *** Nonsignificant or significant at $p \leq 0.05$ and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey-Kramer HSD test ($p = 0.05$).

3.4. Carotenoid Content

Lutein and β-carotene were the principal carotenoid components quantified in the three species of microgreens examined (**Table 4**). Cruciferous microgreens attained higher lutein content than lettuce microgreens, whereas β-carotene was lower in mustard than lettuce and rocket microgreens. Total carotenoids were most abundant in rocket and least abundant in lettuce microgreens. Both carotenoid molecules and total carotenoids were subject to species × DBH interaction. Lutein content in lettuce was non-responsive to DBH treatment, as opposed to mustard, wherein it increased after 12 days of DBH treatment, and rocket, wherein it decreased after 12 days of DBH treatment. A pronounced change in β-carotene content was observed only in rocket microgreens with declining content as DBH treatment increased. The total carotenoids content of lettuce microgreens was unaffected by DBH treatment whereas that of mustard microgreens declined significantly in response to 6-DBH but did not differ between the control and 12-DBH. Rocket microgreens on the other hand demonstrated the greatest response to nutrient deprivation, with 6-DBH and 12-DBH reducing the total carotenoids content by 13.0 % and 27.3 %, respectively.

Table 4. Carotenoid content of three microgreen species (lettuce, mustard, rocket) in response to nutrient deprivation treatment (DBH) applied for 6 or 12 days before harvest. All data are expressed as mean ± standard error, n = 3.

Source of variance	**Lutein** (mg kg^{-1} DW)		**β-carotene** (mg kg^{-1} DW)		**Total carotenoids (mg kg^{-1} DW)**	
Species	***		***		***	
DBH	n.s.		***		***	
Species*DBH	***		***		***	
Lettuce	303.05 ± 3.51	b	442.48 ± 5.31	a	745.53 ± 7.28	b
Mustard	395.71 ± 20.31	a	263.24 ± 16.94	b	658.96 ± 34.23	c
Rocket	405.62 ± 21.98	a	458.42 ± 38	a	864.04 ± 57.8	a
0	381.7 ± 24.42		447.93 ± 41.59	a	829.63 ± 54.6	a
6	354.42 ± 20.89		363.7 ± 41.95	b	718.12 ± 53.95	b
12	368.26 ± 25.09		352.51 ± 22.94	b	720.77 ± 17.68	b
Lettuce,0	303.11 ± 4.34	d	459.55 ± 6.08	b	762.66 ± 4.24	c
Lettuce,6	297.85 ± 5.69	d	426.91 ± 2.65	b	724.76 ± 8.09	cd
Lettuce,12	308.19 ± 8.37	d	440.97 ± 5.17	b	749.16 ± 13.54	c

Mustard,0	390.56 ± 12.81	bc	299.86 ± 13.31	c	690.42 ± 5.48	cd
Mustard,6	330.65 ± 4.28	cd	197.74 ± 3.97	d	528.39 ± 3.38	e
Mustard,12	465.93 ± 13.21	a	292.13 ± 4	c	758.06 ± 9.39	c
Rocket,0	451.44 ± 37.62	ab	584.38 ± 14.09	a	1035.82 ± 50.3	a
Rocket,6	434.75 ± 8.69	ab	466.45 ± 6.79	b	901.2 ± 8.06	b
Rocket,12	330.67 ± 5.13	cd	324.42 ± 11.95	c	655.1 ± 15.06	d

ns, *** Nonsignificant or significant at $p \leq 0.001$, respectively. Different letters within each column indicate significant differences according to Tukey-Kramer HSD test ($p = 0.05$).

3.5. Phenolic Compositi

Eighteen polyphenols were identified and quantified by UHPLC-Q-Orbitrap HRMS in the three microgreen species subjected to nutrient deprivation treatments before harvest (Table 5).

As a proportion of their total phenolic content, the phenolic composition of the three species comprised mainly flavonol glycosides (12.18 – 54.7 %), hydroxycinnamic acids and their derivatives (31.7 – 85.9 %), and flavone glycosides (0.2 – 13.5 %). Phenolic acids were most concentrated in lettuce microgreens and least concentrated in mustard microgreens. Conversely, mustard microgreens were most abundant in flavonol and flavone glycosides. Total phenolic content ranged 715.5 – 1238.0 μg g^{-1} DW across species, being highest in lettuce and lowest in mustard microgreens, whereas a more limited range was encountered between DBH treatments (960.3 – 1053.4 μg g^{-1} DW). Variation in the phenolic components quantified and in the total phenolic content was determined principally by species and only moderately by the DBH treatments. The relative abundance of individual phenolic components varied widely among species. The most pronounced species differentiation was observed for caffeoyl quinic acid, which was at minimal levels (0.48 – 1.69 μg g^{-1} DW) in brassicaceous microgreens and at maximal levels (1055.3 μg g^{-1} DW) in lettuce microgreens, while similar variation was observed for quercetin-3-glucoside. Conversely, quercetin-3-sinapoyl triglucoside and synapoyl-hexose were found at their highest concentration in rocket microgreens and moderately concentrated in mustard microgreens but were respectively non-detectible and at minimal levels in lettuce microgreens.

The phenolic components affected by DBH treatments, as well as the total phenolic content of microgreens, also incurred significant species × DBH interaction that reflects a species-dependent response pattern to DBH treatments. (Table 5). Prominent examples of

this interaction were: caffeoyl quinic acid and quercetin-3-glucoside that climaxed after 6-DBH and 12-DBH treatments respectively in lettuce but remained unaltered in the rest species; ferulic acid that declined progressively with DBH duration in mustard but was unaltered in the rest species; synapoyl-hexose that declined progressively with DBH duration in rocket but was unaltered in the rest of the species. An interaction was also observed with respect to quercetin-3-sinapoyl triglucoside that declined with DBH treatment in rocket, increased in mustard and remained unaltered in lettuce. Interaction was also manifested in respect to the total phenolic content, which declined with DBH treatment in rocket, increased in lettuce and remained unaltered in mustard microgreen.

Table 5. Phenolic composition of three microgreen species (lettuce, mustard, rocket) in response to nutrient deprivation treatment (DBH) applied for 6 or 12 days before harvest. All data are expressed as mean ± standard error, n = 3

	Flavonol glycosides																							
Source of variance	Kaempferol-3-hydroxyferuloyl-sophorotrioside-7-glucoside (µg g^{-1} DW)		Quercetin-3-sophoroside-7-glucoside (µg g^{-1} DW)		Kaempferol-3-glucoside (µg g-1 DW)		Kaempferol 3-diglucoside (µg g^{-1} DW)		Kaempferol-3-sinapoyl-sophoroside-7-glucoside (µg g^{-1} DW)		Kaempferol-3-sinapoyl-sophorotrioside-7-glucoside (µg g^{-1} DW)		Quercetin-3-sinapoyl triglucoside (µg g^{-1} DW)		Quercetin-3-O-rutinoside (rutin) (µg g^{-1} DW)		Quercetin-3-glucoside (µg g^{-1} DW)		Coumaroyl-diglucoside (µg g^{-1} DW)		Isorhamnetin-3-gentiobioside (µg g^{-1} DW)		Total flavonol glycosides (µg g^{-1} DW)	
Species	***		***		***		***		***				***		***		***		***		***		***	
DBH	n.s.		n.s.		n.s.		n.s.		*		n.s.		***		n.s.		***		***		**		***	
Species*DBH	n.s.		***		n.s.		***		n.s.				***		***		***		**		n.s.		***	
Lettuce	n.d.		n.d.		5.58 ± 0.13	a	11.15 ± 0.67	b	n.d.		n.d.		n.d.		16.23 ± 0.89	b	124.7 ± 10.36	a	0.63 ± 0.06	b	n.d.		158.29 ± 11.56	c
Mustard	46.3 ± 0.78	a	10.53 ± 0.56	b	1 ± 0.09	b	18.57 ± 0.39	a	78.46 ± 1.03	a	79.96 ± 1.85		105.1 ± 8.74	b	24.03 ± 0.47	a	0.55 ± 0.08	b	1.54 ± 0.06	a	25.64 ± 1.97	a	391.68 ± 11.54	a
Rocket	0.19 ± 0.02	b	13.8 ± 0.56	a	n.d.		6.91 ± 0.36	c	0.65 ± 0.05	b	n.d.		321.21 ± 17.24	a	13.67 ± 0.85	c	3.04 ± 0.21	b	1.69 ± 0.14	a	8.76 ± 0.41	b	369.92 ± 18.33	b
0	22.86 ± 10.17		12.4 ± 1.59		3.28 ± 1.03		12.01 ± 1.92		38.86 ± 17.07	a	74.7 ± 1.84		216.18 ± 64.46	b	17.86 ± 1.97		29.91 ± 14.36	c	1.08 ± 0.13	b	15.67 ± 3.57	b	291.93 ± 44.52	b
6	22.91 ± 10.15		12.27 ± 0.47		3.21 ± 0.98		12.14 ± 1.6		41.16 ± 18.09	a	80.04 ± 2.13		237.86 ± 50.43	a	18 ± 1.29		44.88 ± 21.44	b	1.2 ± 0.19	b	21 ± 5.11	a	328.51 ± 42.17	a
12	23.96 ± 10.66		11.83 ± 0.55		3.39 ± 1.09		12.48 ± 1.78		38.64 ± 17.07	a	85.13 ± 2.39		185.42 ± 30.43	c	18.08 ± 1.86		53.5 ± 25.78	a	1.58 ± 0.21	a	14.92 ± 3.04	b	299.45 ± 30.08	b
Lettuce,0	n.d.		n.d.		5.57 ± 0.24	a	9.83 ± 0.66	c	n.d.		n.d.		n.d.		14.52 ± 0.61	de	87.16 ± 4.15	c	0.59 ± 0.02	d	n.d.		117.66 ± 2.93	f
Lettuce,6	n.d.		n.d.		5.37 ± 0.18	a	9.99 ± 0.09	c	n.d.		n.d.		n.d.		14.5 ± 0.18	de	130.42 ± 5.21	b	0.48 ± 0.12	d	n.d.		160.77 ± 5.01	e
Lettuce,12	n.d.		n.d.		5.81 ± 0.24	a	13.63 ± 0.31	b	n.d.		n.d.		n.d.		19.67 ± 0.42	c	156.52 ± 3.93	a	0.81 ± 0.08	d	n.d.		196.44 ± 3.64	d
Mustard,0	45.58 ± 1.04		8.88 ± 0.56	c	1 ± 0.14	b	19.4 ± 0.64	a	77.01 ± 1.14		74.7 ± 1.84		72.35 ± 6.17	d	25.57 ± 0.72	a	0.3 ± 0.01	d	1.32 ± 0.06	c	23.57 ± 1.05	b	349.68 ± 8.18	b
Mustard,6	45.56 ± 1.59		11.78 ± 0.85	b	1.06 ± 0.25	b	18.36 ± 0.88	a	81.57 ± 1.93		80.04 ± 2.13		125.37 ± 7.24	c	22.97 ± 0.56	b	0.77 ± 0.13	d	1.59 ± 0.03	bc	31.83 ± 3.64	a	420.9 ± 11.28	a
Mustard,12	47.76 ± 1.45		10.92 ± 0.69	bc	0.96 ± 0.07	b	17.95 ± 0.34	a	76.8 ± 0.62		85.13 ± 2.39		117.57 ± 2.67	c	23.56 ± 0.12	ab	0.57 ± 0.07	d	1.72 ± 0.07	b	21.51 ± 1.63	b	404.47 ± 3.49	a
Rocket,0	0.14 ± 0.02		15.91 ± 0.14	a	n.d.		6.81 ± 0.44	d	0.71 ± 0.05		n.d.		360 ± 7.26	a	13.5 ± 0.95	ef	2.28 ± 0.09	d	1.34 ± 0.07	bc	7.77 ± 0.49	c	408.46 ± 8.09	a
Rocket,6	0.26 ± 0.03		12.75 ± 0.35	b	n.d.		8.07 ± 0.19	cd	0.75 ± 0.09		n.d.		350.36 ± 2.98	a	16.52 ± 0.24	d	3.44 ± 0.12	d	1.53 ± 0.13	bc	10.17 ± 0.29	c	403.85 ± 2.99	a
Rocket,12	0.16 ± 0.02		12.74 ± 0.48	b	n.d.		5.87 ± 0.36	d	0.48 ± 0.01		n.d.		253.26 ± 4.53	b	10.99 ± 0.31	f	3.4 ± 0.23	d	2.21 ± 0.07	a	8.33 ± 0.37	c	297.45 ± 3.37	c

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey-Kramer HSD test ($p = 0.05$).

Table 5. *Cont.*

Source of variance	Hydroxycinnamic acids and derivatives												Flavone glycosides								Total polyphenols	
	Synapoyl-hexose		Ferulic acid		Trisinapoyl-gentiobiose		Disinapoyl-gentiobiose		Caffeoyl quinic acid		Total hydroxycinnamic acids and derivatives		Apigenin-7-*O*-glucoside		Luteolin-7-*O*-glucoside		Total flavone glycosides			
	(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)		(µg g^{-1} DW)	
Species	***		***		***		***		***		***		***		***		***		***	
DBH	***		***		***		n.s.		***		**		n.s.		n.s.		n.s.		***	
Species*DBH	***		***		***		n.s.		***		***		n.s.		n.s.		n.s.		***	
Lettuce	5.35 ± 0.33	c	2.5 ± 0.19	b	n.d.		n.d.		1055.3 ± 31.9	a	1063.15 ± 32.13	a	n.d.		16.51 ± 0.36	b	16.51 ± 0.36	b	1237.95 ± 39.5	a
Mustard	96.86 ± 2.29	b	55.25 ± 2.81	a	23.37 ± 0.41	b	51.01 ± 1.16	b	0.48 ± 0.04	b	226.98 ± 2.06	c	30.97 ± 1.92	a	65.87 ± 5.01	a	96.84 ± 5.63	a	715.5 ± 10.83	c
Rocket	557.84 ± 31.16	a	2.46 ± 0.12	b	43.18 ± 3.71	a	75.62 ± 1.75	a	1.69 ± 0.37	b	680.78 ± 34.68	b	2.32 ± 0.17	b	n.d.		2.32 ± 0.17	c	1053.02 ± 50.89	b
0	252.42 ± 103.5	a	23.2 ± 10.14	a	38.5 ± 7.16	a	63.91 ± 6.16		316.55 ± 158.08	b	660.44 ± 110.91	a	18.35 ± 7.54		42.32 ± 11.58		40.45 ± 15.83	a	992.83 ± 80.41	b
6	220.44 ± 85.49	b	20.62 ± 9.08	b	33.8 ± 5.06	a	61.49 ± 4.72		384.14 ± 191.87	a	688.72 ± 134.57	a	14.68 ± 5.83		39.56 ± 12.58		36.16 ± 14.56	a	1053.38 ± 86.86	a
12	187.19 ± 67.9	c	16.4 ± 7.2	c	27.53 ± 1.8	b	64.54 ± 6.34		356.79 ± 177.63	a	621.75 ± 123.91	b	16.89 ± 6.31		41.7 ± 11.53		39.06 ± 14.75	a	960.26 ± 85.1	b
Lettuce,0	4.3 ± 0.19	e	3.11 ± 0.05	d	n.d.		n.d.		948.58 ± 16.38	c	955.99 ± 16.23	b	n.d.		16.46 ± 0.69		16.46 ± 0.69	b	1090.12 ± 14.6	c
Lettuce,6	5.5 ± 0.43	e	2.53 ± 0.1	d	n.d.		n.d.		1150.67 ± 32.89	a	1158.69 ± 32.69	a	n.d.		15.93 ± 0.51		15.93 ± 0.51	b	1335.39 ± 32.59	a
Lettuce,12	6.24 ± 0.19	e	1.87 ± 0.16	d	n.d.		n.d.		1066.65 ± 26.1	b	1074.77 ± 26.16	a	n.d.		17.13 ± 0.71		17.13 ± 0.71	b	1288.34 ± 22.52	ab
Mustard,0	90.0 ± 2	d	63.75 ± 0.89	a	22.74 ± 0.42	c	50.53 ± 3.11		0.39 ± 0.03	d	227.41 ± 4.03	f	34.78 ± 3.77		68.17 ± 1.19		102.95 ± 4.96	a	680.04 ± 8.94	e
Mustard,6	97.7 ± 2.84	d	56.87 ± 1.79	b	22.66 ± 0.67	c	51.42 ± 1.8		0.45 ± 0.03	d	229.1 ± 4.26	f	27.34 ± 3.13		63.18 ± 15.26		90.51 ± 16.67	a	740.51 ± 14.47	e
Mustard,12	102.9 ± 2.96	d	45.13 ± 1.53	c	24.72 ± 0.24	c	51.07 ± 1.71		0.61 ± 0.01	d	224.43 ± 3.28	f	30.78 ± 2.5		66.26 ± 7.81		97.04 ± 6.29	a	725.95 ± 11.04	e
Rocket,0	662.97 ± 17.56	a	2.75 ± 0.15	d	54.27 ± 2.79	a	77.28 ± 1.03		0.67 ± 0.03	d	797.94 ± 20.9	c	1.93 ± 0.03		n.d.		1.93 ± 0.03	b	1208.32 ± 20.66	b
Rocket,6	558.13 ± 5.98	b	2.45 ± 0.19	d	44.93 ± 1.97	b	71.57 ± 2.55		1.29 ± 0.09	d	678.37 ± 9.58	d	2.03 ± 0.04		n.d.		2.03 ± 0.04	b	1084.25 ± 9.16	c
Rocket,12	452.42 ± 14.93	c	2.18 ± 0.14	d	30.34 ± 2.86	c	78 ± 4.08		3.09 ± 0.21	d	566.03 ± 21.35	e	2.99 ± 0.09		n.d.		2.99 ± 0.09	b	866.48 ± 24.81	d

ns,*,**, *** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Tukey-Kramer HSD test ($p = 0.05$).

4. Discussion

4.1. Yield Characteristics

The lower yielding capacity of rocket microgreens presently demonstrated in comparison to lettuce and mustard microgreens, confirms similar findings from previous studies on the sensitivity of this species to nutrient deprivation (Murphy et al., 2010; El-Nakhel et al., 2021). Variable reduction in fresh yield when grown under complete deprivation of nutrient supplementation (i.e., irrigated only with distilled water), compared to the fertigated control, was demonstrated with microgreens of different brassicaceous species in the study of El Nakhel et al. (El-Nakhel et al., 2021); wherein yield reduction ranged from 7.9% in cabbage to 47.4% in rocket. Analogous species-dependent yield reduction was also reported for different *Brassica* microgreens in response to variable nutrient solution strength (Palmitessa et al., 2020). In the present study however, nutrient deprivation limited to 6-DBH and 12-DBH did not interact with species for microgreens yield, which responded inversely to the extension of treatment duration similarly in all species. El Nakhel et al. (El-Nakhel et al., 2021) also reported a species × fertigation interaction for DM, ranging from no change in cabbage microgreens incurring to 9.8% and 26.8% increase in the DM of Brussel sprouts and rocket microgreens, respectively. The current study demonstrated similar interaction for DM ranging from no change in lettuce microgreens to 29.4% increase in rocket microgreens, underscoring the higher responsiveness of rocket microgreens to nutrient deprivation.

The present results indicate that microgreens fresh yield was dictated primarily by genotype. The deprivation of nitrogen and mineral supply before harvest has had a detrimental effect on fresh yield, however the application of a brief six-day DBH treatment was demonstrated to have a moderate impact, which minimizes economic loss when applied. Moreover, the efficacy of applying brief DBH treatments to increase the DM content of microgreens is promising as a method of potentially enhancing their sensory and phytochemical profile (Rouphael et al., 2018). The genotype-dependent response for DM presently demonstrated warrants however further research into the nature of this interaction before commercial production is targeted on responsive species.

4.2. Canopy Colorimetry

Colorimetric attributes of horticultural products arguably constitute indirect measurements of quality, particularly organoleptic quality and, to an extent, phytochemical content (Pathare et al., 2013). Visual quality is a fundamental constituent of the current regulatory context that defines crop-specific quality standards for horticultural products; moreover, consumers have been increasingly conditioned in perceiving visual traits as indicative of sensory and functional quality (Kyriacou and Rouphael, 2018). The perceived quality of microgreens is strongly related to color since they have been established as garnishes in high gastronomy (Kyriacou et al., 2016a). From the current study it may be inferred that genotype is the main determinant of microgreens' basic color attributes, especially lightness (or darkness) and hue. Notwithstanding their brief growth cycle, nutrient deprivation might potentially undermine color development in microgreens. In terms of overall color intensity (chroma), the current study shows that this effect is not consistent across genotypes and its severity depends on DBH duration, having been manifested only in lettuce subjected to 12-DBH nutrient deprivation. Similarly, El Nakhel et al. (El-Nakhel et al., 2021) reported the absence of fertigation effect on microgreens L* and hue color attributes, and a species × fertigation interaction for chroma with Brussels sprouts and cabbage microgreens incurring non-significant changes as opposed to rocket that declined in chroma by 17.7% when fertigation was replaced by distilled water. In the current study however, loss of greenness (decreasing negative a* values) was registered in all species after 12-DBH nutrient deprivation. It may be inferred therefore that brief nutrient deprivation treatments (< 6 d) can be applied without impacting microgreens' color intensity and the commercial value of the product.

4.3. Nitrate and Mineral Content

The chief source of nitrate in the human diet is the consumption of vegetables, particularly leafy greens, of which rocket, lettuce and spinach combine conduciveness to nitrate accumulation with high consumption thus their maximum nitrate content is stipulated in the European Commission Regulation No 1882/2006 (European Commission Regulation (EC) No 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrates in certain foodstuffs., 2006) (EC, 2006). Although nitrate in itself constitutes a relatively low health hazard, its reaction products and

metabolites (nitrite, nitric oxide and N-nitroso compounds) have been implicated in certain types of oncogenesis and the methemoglobinemia syndrome (EuropeanFood Safety Agency (EFSA)., 2008). Microgreens are generally consumed in limited quantity but nonetheless classify as leafy greens that contribute to the human diet in various forms, from garnishes to smoothies, and their most common genotypes derive from the Brassicaceae, which includes known nitrate hyperaccumulators such as rocket (Kyriacou et al., 2016a; Colla et al., 2018). Moreover, certain wild genotypes [e.g., small burnet (*Sanguisorba minor*); wild mustard (*Sinapis arvensis*) and common dandelion (*Taraxacum officinale*)] that comprise a repository of interest for the growing microgreens industry, have in previous studies demonstrated nitrate hyper-accumulating capacity with concentrations ranging 2834 – 7129 mg kg^{-1} (Lenzi et al., 2019).

In the current study, rocket microgreens attained three-fold higher nitrate concentration than lettuce and mustard. The latter two attained similar nitrate content as previously found in coriander, kohlrabi, pak choi, basil, beet and rapini microgreens sustained with nutrient solutions on various substrates (Brazaitytė et al., 2015; Di Gioia et al., 2017; Kyriacou et al., 2020). The detected levels were in all cases, including rocket microgreens, well below the tolerance maxima stipulated in EC Reg. No 1882/2006 (European Commission Regulation (EC) No 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrates in certain foodstuffs., 2006). Our current results moreover corroborate previous studies that demonstrated a comparably lower accumulation of nitrate in microgreens than their mature counterparts (Pinto et al., 2015; El-Nakhel et al., 2020; Ferrón-Carrillo et al., 2021). The nutrient deprivation treatment was demonstrated overall as effective in reducing the nitrate content of all genotypes studied. Analogous reduction of leaf nitrates was found in hydroponically cultivated cardoon leaves subjected to 15-day nutrient deprivation prior to harvest (Borgognone et al., 2016). The duration of the nutrient deprivation treatment required for effective reduction of nitrate levels differed between genotypes, with the nitrate hyperaccumulating species, such as rocket, being more responsive to DBH treatments than other species. It is worth reiterating that rocket microgreens not deprived of fertigation attained 3.6- and 4.9-fold higher nitrate content than mustard and lettuce, respectively; however, six- and twelve-day nutrient deprivation resulted in dramatic reduction on nitrate by 72.9% and 90.3%, respectively. By

decreasing the strength of Hoagland solution, El Nakhel et al. (El-Nakhel et al., 2019) observed a similar genotype-dependent response in the total N content of green vs. red Salanova lettuce, with the content remaining unaltered in the former but decreasing in the latter. As presently demonstrated across species, a brief six-day nutrient deprivation constitutes a significant tool for depleting nitrate deposits prior to harvesting microgreens, thus increasing food safety in terms of antinutritive agents such as nitrate.

The role of minerals in human nutrition is critical in supporting optimum development while maintaining body homeostasis and metabolic functionality, as well as in preventing nutritional deficiencies associated with physiological disorders (Gharibzahedi and Jafari, 2017). Fruits and vegetables contribute substantially as dietary sources of minerals, with their percentage contribution towards K, Na, Ca, Mg and P dietary requirements accounting respectively for 35, 11, 7, 24 and 11% of total requirements (Levander, 1990). Analytical data on the mineral composition of microgreens is yet limited but the role of genotype as key determinant of mineral composition has been highlighted and is reiterated by the current results. Notwithstanding genotype differences, the current work corroborates previous studies that reported microgreen mineral content in declining order as K> Ca> Mg > S (Xiao et al., 2016; Kyriacou et al., 2019a; Palmitessa et al., 2020). However, data comparison between studies is hampered by variation in experimental conditions that may effectively modulate the mineral content of microgreens such as, nutrient solution composition, light quality, intensity and photoperiod, growth substrate and harvest maturity (Kyriacou et al., 2016a)

The present work highlighted that the P and K content of microgreens is highly responsive to nutrient deprivation across genotypes with both minerals having demonstrated moderate reduction, as opposed to the divalent ions Ca^{++} and Mg^{++} that were non-responsive. This outcome might be attributed to the mineral composition of the peat-based substrate that apparently included adequate levels of Ca^{++} and Mg^{++} to support the brief growth cycle of microgreens under nutrient deprivation conditions. We have previously demonstrated that natural fiber and synthetic substrates differing in physicochemical constitution may modulate the mineral and indirectly the phytochemical composition of microgreens (Kyriacou et al., 2020). Moreover, it has been demonstrated that nutrient composition of typical peat-based substrates may support satisfactory growth of microgreens without

supplying additional nutrients, which renders their cultivation readily accessible to non-professionals (Pannico et al., 2020). Considering that microgreens are marketed not only as flavorful and colorful condiments or garnishes but also as potent sources of phytochemicals and minerals, the impact of depriving nutrients (solution- or substrate-provided) on the mineral content of microgreens is presently underscored. Regarding the genotype-dependent response to nutrient deprivation observed for S content, the higher responsiveness of brassicaceous species most likely relates to their increased demands for S implicated in glucosinolate biosynthesis (Neugart et al., 2018).

4.4. Carotenoid Content

The content of lutein and β-carotene, the major carotenoid compounds found in microgreens, contributes to their bioactive value since they constitute lipophilic antioxidants owing to the light-absorbing and ROS-quenching properties of their polyene chains (Young and Lowe, 2001). Lutein dietary uptake or supplementation has been linked to protection against oxidative damage and macular degeneration, while β-carotene is an essential precursor of vitamin A required for development and effective function of the immune and optical system (Kvansakul et al., 2006). Based on the results of the current study, we can conclude that carotenoid levels in microgreens are primarily dictated by genotype, which supports previous studies on the carotenoid content of microgreens of different species or cultivars grown under variable conditions (Xiao et al., 2012; Brazaitytė et al., 2015; Kyriacou et al., 2020; Ferrón-Carrillo et al., 2021). Moreover, response to nutrient deprivation with respect to carotenoid composition is genotype-specific, with certain species, such as lettuce, being non-responsive and others, such as rocket, being depleted of carotenoid content with increasing treatment duration. Genotype-dependent decline in carotenoids was also reported in lettuce grown hydroponically under decreasing concentrations of nutrient solution (El-Nakhel et al., 2019). It seems plausible that depletion of carotenoids with nutrient deprivation is more pronounced in carotenoid-rich species. However, even in carotenoid-rich species the depletion of carotenoids during the first six days of DBH treatment seems moderate, with 14.9% and 37.6% reduction taking place between days 0 – 6 and 6 – 12, respectively. Therefore, brief DBH treatments are likely to cause moderate depletion of carotenoids in carotenoid-rich species, which arguably would not compromise significantly the bioactive value of the product.

4.5. Phenolic Composition

The phenolic composition of microgreens appraised through UHPLC-Q-Orbitrap HRMS was dominated by flavonol glycosides in mustard and by hydroxycinnamic acids in lettuce and rocket. Quercetin and kaempferol glycosides were the flavonol glycosides most abundant in brassicaceous microgreens, whereas lettuce microgreens were steeped in caffeoyl quinic acid. The current findings largely corroborate previous reports on microgreens' composition and confirm the wide variation in phenolic components encountered even among related brassicaceous genotypes (Sun et al., 2013; De la Fuente et al., 2019; Klopsch et al., 2019). It is further demonstrated that nutrient deprivation before harvest may impact significantly the phenolic constituents of microgreens and their total phenolic content. However, the effect of DBH treatment on most phenolic components and on the total phenolic content seems highly species-dependent. Intriguingly, lettuce microgreens where the most positively responsive to nutrient deprivation as both 6-DBH and 12-DBH treatments increased the total flavonol glycosides, total hydroxycinnamic acids, and the total phenolic contents. Mustard microgreens were moderately responsive, as their flavonol glycoside increased in response to 6-DBH and 12-DBH treatments but not their hydroxycinnamic acids, and total phenolic contents. Finally, rocket microgreens were the most negatively responsive to nutrient deprivation, which resulted in the reduction of their content in flavonol glycosides, hydroxycinnamic acids and total phenols. It is therefore apparent that aside from its utility in reducing the nitrate levels of microgreens, DBH treatments mimic *eustress* applications in modulating the products of the phenylpropanoid pathway (El-Nakhel et al., 2019), particularly when they extend beyond 6-DBH. The positive response to DBH treatments exhibited by certain species, such as lettuce, which incurred significant increase in its flavonol glycoside and hydroxycinnamic acid components, might render nutrient deprivation an important tool for enhancing the bioactive value of microgreens. This prospect is encouraging in light of previous findings concerning the analgesic and anti-inflammatory activities of hydroxycinnamic acids (Alfaifi et al., 2020), and the *in vitro* antiproliferative, and *in vivo* antioxidant and anti-inflammatory activity of quercetin derivatives (Tajik et al., 2017;Whitaker and Stommel, 2003). Such applications however, warrant further research to determine the responsiveness of targeted species to nutrient deprivation treatments, as well as to link changes in phenolic composition

to variation in the sensory and *in vivo* bioactive value of microgreens (Granato et al., 2018). It is nonetheless noteworthy that a brief six-day nutrient deprivation before harvest has had overall a moderate impact or no effect on the phenolic composition of microgreens across species, thus it can be applied to reduce nitrate residual in microgreens without dramatically shifting the phenolic composition and its potential implications for the bioactive value.

5. Conclusions

Nutrient deprivation was an effective method for reducing the nitrate content of microgreens, however effective treatment duration differed between species. Decline in nitrate content is more precipitous in hyperaccumulating species such as rocket, which incurred 72.9% and 90.3% reduction of nitrate content after 6-DBH and 12-DBH treatments, respectively. The present work also demonstrated that brief (≤ 6 days) nutrient deprivation treatment before harvest can be applied across species with moderate or no impact on the phenolic, carotenoid and mineral composition of microgreens. Such brief DBH applications also seem to have limited impact on microgreens' yield and color intensity and therefore on the commercial value of the product. It can therefore be applied to reduce nitrate levels in microgreens without dramatically shifting their key secondary metabolite content and its potential implications for bioactive value.

Chapter 7: General Conclusions

The relatively brief growth cycle required for microgreens to reach harvest maturity renders genotype selection a key component for this expanding new industry. Important compositional differences were presently characterized across microgreens from 13 species and five botanical families. Nitrate hyper-accumulator microgreens were identified that warrants preharvest measures to suppress nitrate content. Across species, K was the most abundant macro-mineral, followed by Ca, P, Mg, S and Na. Genotypic differences in Na, K and S concentrations were wide while variation in P, Ca and Mg was narrower. Antioxidant capacity assayed in vitro was highest in brassicaceous microgreens. The levels of ascorbic acid present in microgreens were higher than corresponding levels in sprouts, plausibly owing to the presence of photosynthetic hexose precursors absent from sprouts. Genotypic variation in pigmentation was also expressed in terms of chlorophyll and carotenoid concentrations. Lamiaceae microgreens exhibited comparatively higher phenolic content, notwithstanding significant varietal differences. Moreover, alternative phenolics-rich species of microgreens, such as coriander from the Apiaceae were for the first time identified. Qualitative and quantitative determination of phenolic profiles demonstrated the predominance of flavonol glycosides, with the O-glycosides of kaempferol showing more species-related distribution. Principal Component Analysis revealed that the clustering of phenolic profiles reflected microgreens' botanical taxonomy with relative consistency. Such information is critical for selecting new species/ varieties of microgreens that satisfy demand for both taste and health.

Further to genotype selection, the targeted modulation of microgreens secondary metabolism through select spectral bandwidths was assessed as a tool to produce phytochemically-enriched microgreens of high functional quality and nutritive value. Analytical data on microgreens' response to different light spectra constitutes a valuable resource for designing future crop-specific spectral management systems. Thus, variation in productivity, nutritive and functional quality of novel microgreens (amaranth, cress, mizuna, purslane) was examined in response to select spectral bandwidths (red, blue, blue-red).

Growth parameters dependent on primary metabolism were found most favored by blue-red light's efficiency in activating the photosynthetic apparatus. Nitrate accumulation was higher under monochromatic light owing to the dependency of nitrite reductase on the light-driven activity of PSI, most efficiently promoted by blue-red light. Mineral composition was mostly genotype-dependent, however monochromatic red and blue lights increased K and Na and decreased Ca and Mg concentrations. Lutein, β-carotene, and lipophilic antioxidant capacity were generally increased by blue-red light putatively due to the coupling of heightened photosynthetic activity to increased demand for protection against oxidative stress. Finally, the general response to light treatments was a decrease in polyphenolic constituents, particularly flavonol glycosides, and total polyphenols under blue-red light. Notwithstanding that genotype specificity underlies some of the responses to light treatments summarized above, the current work highlights how selection of genetic background combined with effective light management can drive the production of microgreens with superior functional quality.

The choice of growth substrate is critical for the production of high-quality microgreens. Therefore, understanding how the physicochemical properties of natural fiber (agave fiber, coconut fiber and peat moss) and synthetic substrates (capillary mat and cellulose sponge) impact the growth and yield attributes, the nutritive and phytochemical composition and the antioxidant potential of select microgreen species (coriander, kohlrabi and pak choi) wan imperative and novel next step in the present line of research. A key finding of this work, which advances our understanding of the current and future literature on microgreens production and potential bioactive value, is that substrates which combine optimal physicochemical properties, such as peat moss, tend to promote faster growth and higher fresh yields that favor high production turnover; however, this is achieved at the expense of reduced phytochemical content, foremost of polyphenols. Therefore, controlled stress applications (e.g., osmotic stress) on microgreens growing on such media warrants investigation as a means of enhancing phytochemical composition without substantial compromise in crop performance and production turnover. Substrates promoting fast growth (e.g., peat moss) also tend to promote nitrate accumulation in microgreens, especially in brassicaceous species that are known nitrate hyperaccumulators. Therefore, nitrate

deprivation practices should be considered for microgreens grown on such substrates in order to minimize consumer exposure to nitrates.

Although microgreens have become acclaimed as novel gastronomic ingredients that combine visual, kinesthetic and bioactive qualities, the definition of the optimal developmental stage for their harvesting remains fluid. The ontogenetic stages for harvesting microgreens range from the cotyledonary stage to the emergence of the second true leaf. Their superior phytochemical content against their mature counterparts fueled the subsequent work hypothesis that significant changes in their compositional profile likely take place during the brief interval of ontogeny from the appearance of the first (S1) to the second true leaf (S2). Elucidating this hypothesis will contribute towards the standardization of harvest maturity for the microgreens industry. Microgreens of four brassicaceous genotypes (Komatsuna, Mibuna, Mizuna and Pak Choi) thus grown under controlled conditions, harvested at S1 and S2. They were appraised for yield traits and subsequently examined for mineral, volatile organic compounds, polyphenols, ascorbate as well as hydrophilic and lipophilic pigment concentrations. Analysis of compositional profiles revealed genotype as the principal source of variation for all constituents. The absence of significant growth stage effect on many of the phenolic components identified is consistent with previous findings that post-germination differences in phenolic composition between S1 microgreens and baby leaves are minimal. The response of mineral and phytochemical composition and of antioxidant capacity to growth stage was also limited and largely genotype-dependent. It is, therefore, questionable whether delaying harvest from S1 to S2 would significantly improve the bioactive value of microgreens while the cost-benefit analysis for this decision must be genotype-specific. In terms of yield, the lower-yielding genotypes (Mizuna and Pak Choi) registered higher relative increase in fresh yield between S1 and S2, compared to the faster-growing and higher-yielding genotypes. Although the optimal harvest stage for specific genotypes must be determined considering the increase in yield against reduction in crop turnover, harvesting at S2 seems advisable for the lower-yielding genotypes.

As reiterated above, microgreens constitute rudimentary leafy greens that impart gastronomic novelty and sensory delight, but are also packed with nutrients and phytochemicals. As such, they comprise an upcoming class of functional foods. However,

apart from bioactive secondary metabolites, microgreens also accumulate antinutritive agents such as nitrate, especially under conducive protected cultivation conditions. As stated above, commercially favorable substrates such as peat moss promote fast growth but also tend to promote nitrate accumulation in microgreens, warranting nitrate deprivation practices in order to minimize consumer exposure to nitrates. In this perspective, nutrient deprivation before harvest (DBH) was examined as a plausible strategy, applied by replacing nutrient solution with osmotic water for six and twelve days, on different species (lettuce, mustard and rocket) of microgreens. DBH impact on major constituents of the secondary metabolome, mineral content, colorimetric and yield traits was appraised. Nutrient deprivation was found effective in reducing nitrate content, however effective treatment duration differed between species with decline being more precipitous in nitrate hyperaccumulating species such as rocket. DBH interacted with species for phenolic constituents. It increased the phenolic content of lettuce, decreased that of rocket and did not affect mustard. Further research to link changes in phenolic composition to the sensory and in vivo bioactive profile of microgreens might be warranted. However, it may be safely concluded that brief ($\leq$ 6 days) DBH can be applied across species with moderate or no impact on the phenolic, carotenoid and mineral composition of microgreens. Such brief nutrient deprivation applications also have limited impact on microgreens' yield and colorimetric traits hence on the commercial value of the product. They can therefore be applied for reducing microgreen nitrate levels without significantly impacting key secondary metabolic constituents and their potential bioactive role.

Through step-wise examination and appraisal of critical preharvest factors – ranging from genotype and substrate selection, to spectral management, ontogenetic stage at harvest and nutrient deprivation schemes – the current project contributes to the advancement of our understanding on the role and potential utility of these factors in configuring microgreens' yield, sensory, safety, nutritive and bioactive profile.

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