Diversification of health-promoting phytochemicals in radish (*Raphanus raphanistrum*) and kale (*Brassica oleracea*) micro-greens using high light bio-fortification

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ABSTRACT

Background: Fruits and vegetables contain significant amounts of biologically active phytochemicals (such as polyphenols, glucosinolates, phytoestrogens, and carotenoids, amongst others), which have associated with human health and nutrition. Numerous bio-fortification strategies are employed to enhance the nutritional profile of plant-based foods to address and minimize the severe outcomes of malnutrition.

Methods: Using an established high light-induced bio-fortification strategy, we aimed to augment the accumulation of health-promoting phytochemicals in a selection of Brassica micro-greens (kale and radish). High throughput tandem mass spectrometry was used to identify the differential accumulation of phytochemicals and subsequently determined their antioxidant capacity. Using a classical DNA protection assay, we demonstrated that human genomic DNA could be protected from oxidative stress.

Results: We report here on the potential link between the increased phytochemicals, total antioxidant, capacity and potential consequent role in human DNA protection.

Conclusion: Bio-fortification implemented as a future strategy could enhance the phytochemical profile and consequent antioxidant potential for the development of functional foods and food supplements.

BACKGROUND

Basic nutrition is the cornerstone of good health and requires an adequate intake of foods rich in macro- (carbohydrates, proteins,   and fats) and micro-nutrients (vitamins and minerals). Functional foods (foods that provide health benefits beyond basic nutrition) contain many biologically active compounds known to be positively associated to human health. These include terpenes [1, 2], polyphenols [3, 4], glucosinolates [5, 6], phytoestrogens [7, 8, 9] and carotenoids [10, 11]. Collectively termed phytochemicals, these biologically active compounds form part of a plant's secondary metabolite profile and often accumulate as part of global stress response (both abiotic and biotic) mechanisms [12, 13, 14].

Nutrition- and health-based research actively seeks specific compounds in the diet-disease relationship to guide consumers toward an optimal disease-prevention diet. Increasing evidence suggests that Brassica vegetables (also known as cruciferous vegetables) and leafy greens (including broccoli, kale, mustard greens, rocket, radish, cabbage, cauliflower amongst many others) are potentially effective at protecting against cardiovascular diseases and some cancers, owing to their high content of glucosinolates and isothiocyanates [15, 16]. Despite Brassica species being well described to accumulate several other important phytochemicals as well, a sustained and diverse diet (Brassica-enriched) is required for health beneficial effects [17].

While all plants accumulate a diverse variety of phytochemicals, micro-greens (MGs, immature plants with 2-4 true leaves) hyper-accumulate phytochemicals (up to 100 times more than mature plant [18, 19]. Consequently, MGs are generally considered nutritionally superior. The study of MGs is an emergent research field   focused on   their health and potential disease prevention benefits [20, 21]. As small edibles (with 2-6 true leaves), MGs are harvested 10-25 days after germination. During this adaptive growth stage, they accumulate substantial amounts of vitamins, minerals, and a diverse range of phytochemicals. Comparatively, older (environmentally adapted) plants do not display this enhanced nutritive profile and rely on alternate stress response pathways [22, 23, 24, 25, 26].

Brassica MGs have been very popular in the culinary world and   often used for enhanced sensory purposes. More recently, several Brassica MGs have been recognised for their enriched phytochemical and nutritional qualities [27, 28]. Consequently, the large number   of vegetables required daily could be condensed into smaller dietary portions containing significantly more health beneficial phytochemicals. MGs serve as an ideal target for bio-fortification strategies whereby phytochemical profiles could be altered during plant growth, using abiotic environmental stress stimuli. Because these secondary metabolites accumulate as part of stress response-mechanisms, we demonstrate in this study the effectiveness of high light (HL) as a bio-fortification strategy to enhance the phytochemical profile of MGs for radish (*Raphanus raphanistrum*) and kale (*Brassica oleracea*), two commonly consumed Brassica species.

MATERIALS AND METHODS
Plant material and growth conditions
Radish and kale seeds (*Raphanus raphanistrum* subsp. sativus*, Brassica oleracea* var. sabellica) were obtained from a commercial seed supplier (Seeds for Africa, South Africa). Subsequent to

stratification (24 h, 4 ºC), plants were propagated to MG stage (2-6 true leaves) under (i) controlled growth conditions (16 h light, 70 µmol photons m^{-2} s^{-1}, 22 ºC, 8 h dark, 22 ºC, 60% relative humidity) and (ii) high light intensity (270 µmol photons m^{-2} s^{-1}). All plants were maintained and propagated on peat disks (Jiffy™ no.7, South Africa).

Total starch and metabolite extractions

Total starch was extracted from whole, lyophilized MG tissue (50 mg) using the Total Starch HK Assay kit (Megazyme International Ireland Ltd.) and quantified using the online tool Mega-Calc™ software tool.

Metabolites were extracted with acetonitrile from whole, lyophilized MG tissue (300 mg), as previously described [18, 29]. Prior to further analysis, polyphenolic extracts were lyophilised and desalted as previously described (omitting the Polyklar AT; [30]).

Ascorbic acid and antioxidant capacity measurements

Ascorbic acid was quantified in lyophilised micro-green tissue (500 mg) using an Ascorbic Acid Assay kit (MAK074, Sigma-Aldrich, South Africa), according to manufacturer's instructions. Similarly, total antioxidant capacity was determined on lyophilised, micro-green tissue (500 mg) as compared against a Trolox standard, using the Total Antioxidant Capacity Assay kit (MAK187, Sigma-Aldrich, South Africa), according to manufacturer's instructions.

DNA damage assays

DNA damage assays were performed as previously described [31]. Human genomic DNA (0.5 µg/µL; cat. no. 11 691 112 001, Roche, Sigma, South Africa) was diluted in phosphate buffer (50 mM, pH 7.4) and damaged using Fenton's reagent (1 mM $FeSO_4$, 0.1 mM H_2O_2). Samples were subject to heat (37°C, 30 min),   and DNA damage analyz   ed via electrophoresis (0.8% agarose). Damaged DNA was observed as a loss of integrity (smear). DNA protection was assessed by the addition of lyophilized MG extracts (diluted with water to concentrations of 1000, 100 and 10 ⌈g ml^{-1}) prior to the addition of Fenton's reagent [31]. DNA protection was observed as retained integrity (no smear). Trolox, an analogue of vitamin E, was used as control.

Tandem mass spectrometry (LC-MS/MS) analyses and phenolic compound identification

LC-MS/MS analyses were performed, as previously described [18], with a Waters Synapt G2 quadrupole time-of-flight mass spectrometer (Waters Corporation, Milford, MA, USA) equipped with a Waters Acquity UPLC. Samples were separated on a Waters UPLC BEH C18 column (2.1 x 100 mm; 1.7 µm) at a flow rate of 0.3 ml/min at 55 ºC. Solvent A consisted of 0.1% formic acid in water,   and solvent B was 0.1% formic acid in acetonitrile. The mobile phase gradient was initiated at 100% solvent A for 1 min and linearly reduced to 28% solvent A over 22 min. Subsequently, the mobile phase was changed to 40% solvent B over 50 s followed by a wash step in 100% solvent B before the column was re-equilibrated to the initial conditions for 4 min. Electrospray ionization was applied in the negative mode,   and the scan range was either from m/z 150-1500 (high collision energy scan) or   m/z 40-1500 (low collision energy scan). The photo   diode array detector was set to scan from 220-600 nm. The capillary voltage was set at either 6 V (low collision energy scan from) or 30-60 V (high collision energy scan), the cone voltage was 15 V, the source temperature 120 ºC and the desolvation temperature was 275 ºC. The

desolvation and cone gas (nitrogen) flows were 650 L/h and 50 L/h, respectively. Sodium formate was used for calibration, and leucine encephalin was infused in the background as lock mass for accurate mass determinations. Metabolites were monitored using their deprotonated quasi-molecular ions. Base peak chromatograms (extracted for NL and HL, respectively) were overlaid to identify the compounds that were differentially accumulating. Compounds were predicted using the Metabolomics workbench (www.metabolomicsworkbench.org; [32]). The database was searched, using the m/z mass obtained from total ion chromatograms, with parameters set to the negative ion [M-H]- mode and a mass tolerance of +/- 0.2 m/z. Tentative identification was based on the accurate mass and fragment ions of the specific peaks compared to literature.

Statistical analyses

All data acquired in this study are the results of three independent experiments, expressed as means $\pm$ standard error. Statistical significance was determined by a two-   tailed t-test ($p < 0.055$), using the control group (normal light) as a comparison. Statistical analysis was performed with GraphPad Prism (version 7.0).

RESULTS

High light induces structural adaptations, increases starch accumulation and alters the accumulation of ascorbic acid

Radish and kale, propagated to MG stage under HL conditions, displayed structural phenotypic responses when compared to normal light (NL) conditions (Figure 1).
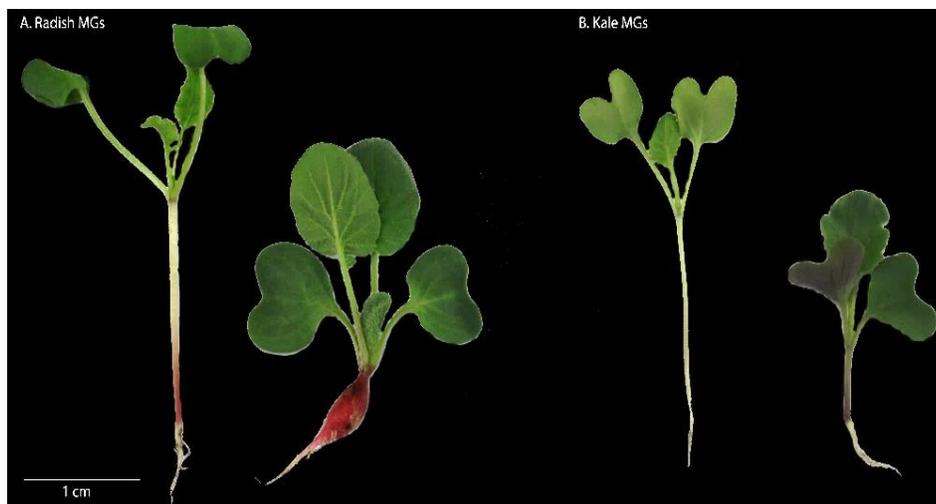

Figure 1: Comparison between phenotypic responses occurring in (A) radish and (B) kale MGs grown under HL**.** Phenotypic differences included stunted stem development, broader leaf surface area, purple coloration, and storage root development (the latter only applying to radish).

Both radish and kale MGs displayed stunted stem elongation and larger leaves under HL conditions when compared to NL conditions. The exposure to HL further induced purple coloration in the leaves of kale but not in radish. Radish MGs, however, showed the rapid development of storage roots. Total starch content increased significantly in both radish and kale MGs. Radish accumulated almost 9-fold higher total starch, under HL when compared to NL

conditions (191.9 ± 30.1 mg g^{-1} DW and 20.9 ± 5.2 mg g^{-1} DW for HL and NL, respectively; Figure 2). Kale accumulated almost 3-fold higher total starch, under HL when compared to NL conditions (106.2 ± 18.2 mg g^{-1} DW and 35.7 ± 15.4 mg g^{-1} DW for HL and NL, respectively; Figure 2). Ascorbic acid content decreased significantly in radish MGs, almost 3-fold lower, under HL when compared to NL conditions (0.37 ± 0.02 mg g^{-1} DW and 0.15 ± 0.01 mg g^{-1} DW for HL and NL, respectively; Figure 3). Ascorbic acid content did not change significantly in kale MGs (0.04 ± 0.03 mg g^{-1} DW and 0.09 ± 0.01 mg g^{-1} DW for HL and NL, respectively; Figure 3).
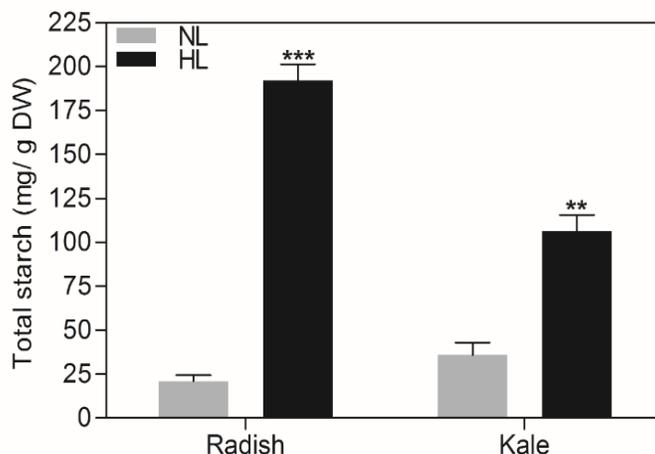

Figure 2: Total starch was compared between radish and kale MGs propagated under normal and high light conditions. Total starch content in lyophilized tissue was determined from three independent experiments, using pooled samples of micro-greens (approximately 200 plants per replicate). Statistical significance is indicated by stars as determined by a two tailed t-test, using NL as the comparison control (radish HL, **p = 0.0052; kale HL, ***p = 0.0039). NL, normal light; HL, high light.
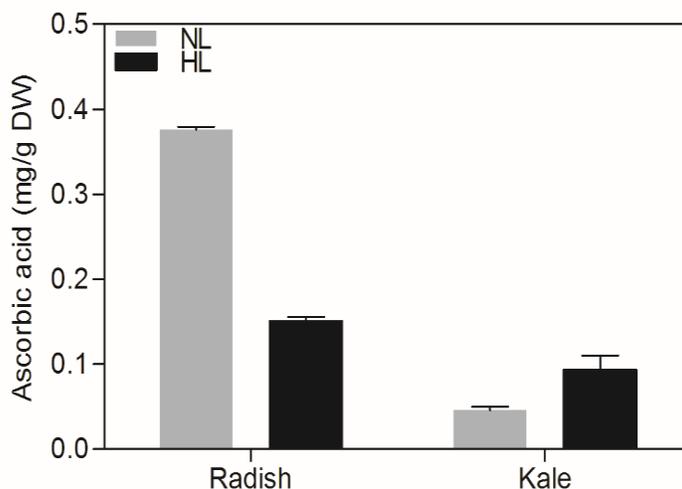

Figure 3: Accumulation of ascorbic acid was compared between radish and kale MGs propagated under normal and high light conditions. Ascorbic acid content in lyophilized tissue was determined from three independent experiments, using pooled samples of micro-greens (approximately 200 plants per replicate). Statistical significance is indicated by stars as determined

by a two tailed t-test, using NL as the comparison control (radish HL **p ≤ 0.0012; kale HL, p = 0.0741). NL, normal light; HL, high light.

High light positively influences total antioxidant capacity

Acetonitrile extracts from radish, and kale MGs were analyzed further for their total antioxidant capacity (TAC), expressed as Trolox (vitamin E) equivalents. The TAC increased significantly in extracts from both radish and kale MGs grown under HL when compared to NL. The TAC in extracts from radish MGs increased almost 1.7-fold under HL when compared to NL conditions (4.6 ± 0.6 mg g^{-1} DW and 2.6 ± 0.7 mg g^{-1} DW for HL and NL, respectively; Figure 4). The TAC in extracts from kale MGs increased almost 2.5-fold under HL when compared to NL conditions (9.2 ± 1.8 mg g^{-1} DW and 3.6 ± 0.5 mg g^{-1} DW for HL and NL, respectively; Figure 4).
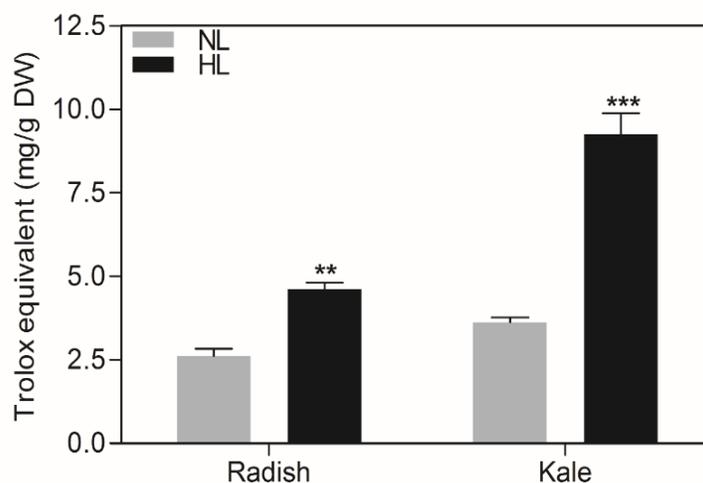

Figure 4: Total antioxidant capacity (TAC) was compared between radish and kale MGs propagated under normal and high light conditions. TAC in lyophilized tissue was determined from three independent experiments, using pooled samples of micro-greens (approximately 200 plants per replicate) and expressed as Trolox equivalents. Statistical significance is indicated by stars as determined by a two- tailed t-test, using NL as the comparison control (radish HL, **p = 0.0052; kale HL, ***p = 0.0008). NL, normal light; HL, high light.

DNA damage/protection assays on human genomic DNA were conducted using Fenton's reagent (H_2O_2 induces oxidative stress), in the presence and absence of various concentrations of MG extracts to assess whether extracts from radish and kale MGs were efficient as antioxidants (Figure 5). The DNA that was incubated without (no oxidative damage) or with Fenton's reagent (oxidative damage) and analyz ed using electrophoresis, with the DNA being visualised in an agarose gel as bright and intact (undamaged) or lighter smeared (damaged) bands, respectively. The DNA that was pre-incubated with extracts from radish and kale MGs (at concentrations of 1000, 100, and 10 ⌈g ml^{-1}, respectively) and incubated with Fenton's reagent was assessed. No DNA protection was observed for the lowest concentration (both extracts, 10 ⌈g ml^{-1}). Varying degrees of DNA protection was observed at higher concentrations (both extracts, 1000 and 100 ⌈g ml^{-1}). Comparatively, the extract from radish MGs grown under NL exhibited the greatest

DNA protection at a concentration of 100 ⌈g ml⁻¹. At concentrations of 1000 ⌈g ml⁻¹ extracts from both radish and kale under NL and HL exhibited DNA protection abilities.
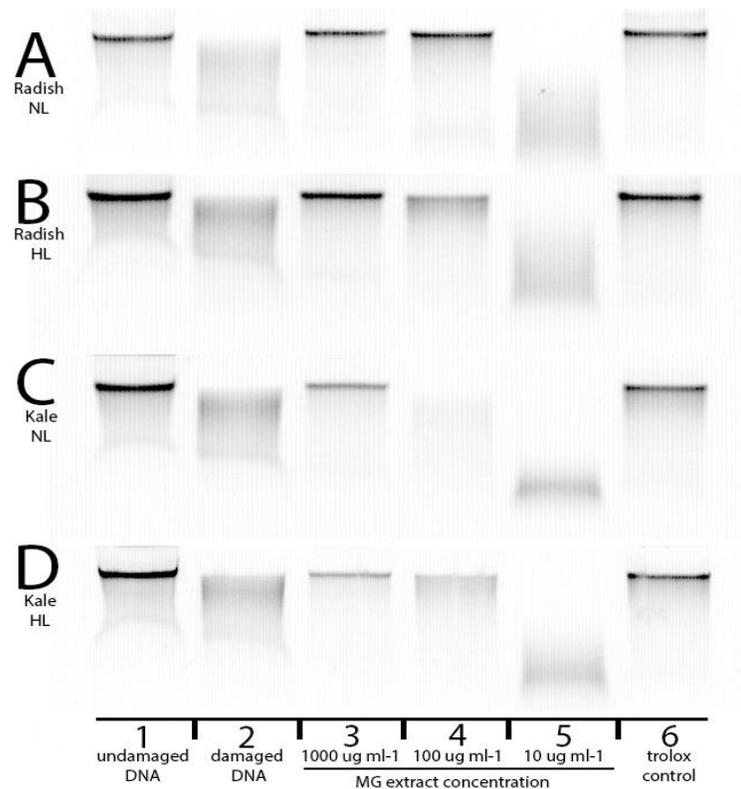

Figure 5: DNA damage/protection assays compared between radish and kale MGs propagated under normal (A, radish and C, kale) and high light (B, radish and D, kale). Human genomic DNA was treated with Fenton's reagent to induce oxidative stress. Lane 1, undamaged DNA; Lane 2, damaged DNA; Lane 3 -5, DNA pre-treated with either 1000, 100 or 10 g ml MG extract respectively; Lane 5, Trolox (control). NL, normal light; HL, high light.

Comparison of phytochemicals accumulating in response to high light stress

Tandem mass spectrometry analyses (LC-MS/MS) was conducted on extracts from radish and kale MGs to identify specific compounds and/or groups of compounds that accumulated in response to HL (compared to NL). The extracts from radish MGs grown under HL differentially accumulated (i) carbohydrates, (ii) coumarin and/or cinnamic acids, (iii) proanthocyanidins, and (iv) flavonoid glycosides (Table 1, Figure 6). The compounds were grouped according to their mass and structure predictions from which specific compounds for proanthocyanidins (Epigallocatechin-(4beta->8)-epicatechin-3-O-gallate ester) and glucosinolates (4-Hydroxy-3-indolylmethylglucosinolate) were identified.

The extracts from kale MGs grown under HL differentially accumulated (i) phenolic acids, (ii) amino- and sulfated- carbohydrates, and (iii) flavonoids (Table 1, Figure 6). The compounds were grouped according to their mass and structure predictions from which specific compounds for flavonoids (3,5,7-Tris(acetyloxy)-2-[4-(acetyloxy)-3-hydroxyphenyl]-4H-1-benzopyran-4-one), amino oligosaccharides (N-acetyl-D-galactosaminyl-(1->4)-(N-acetyl-D-galactosaminyl)-(1->3)-N-acetyl-D-galactosaminitol) and carbohydrate sulfates (alpha-Neup5Ac-(2->3)-beta-D-Galp-(1->3)-alpha-D-GalpNAc6S) were identified.
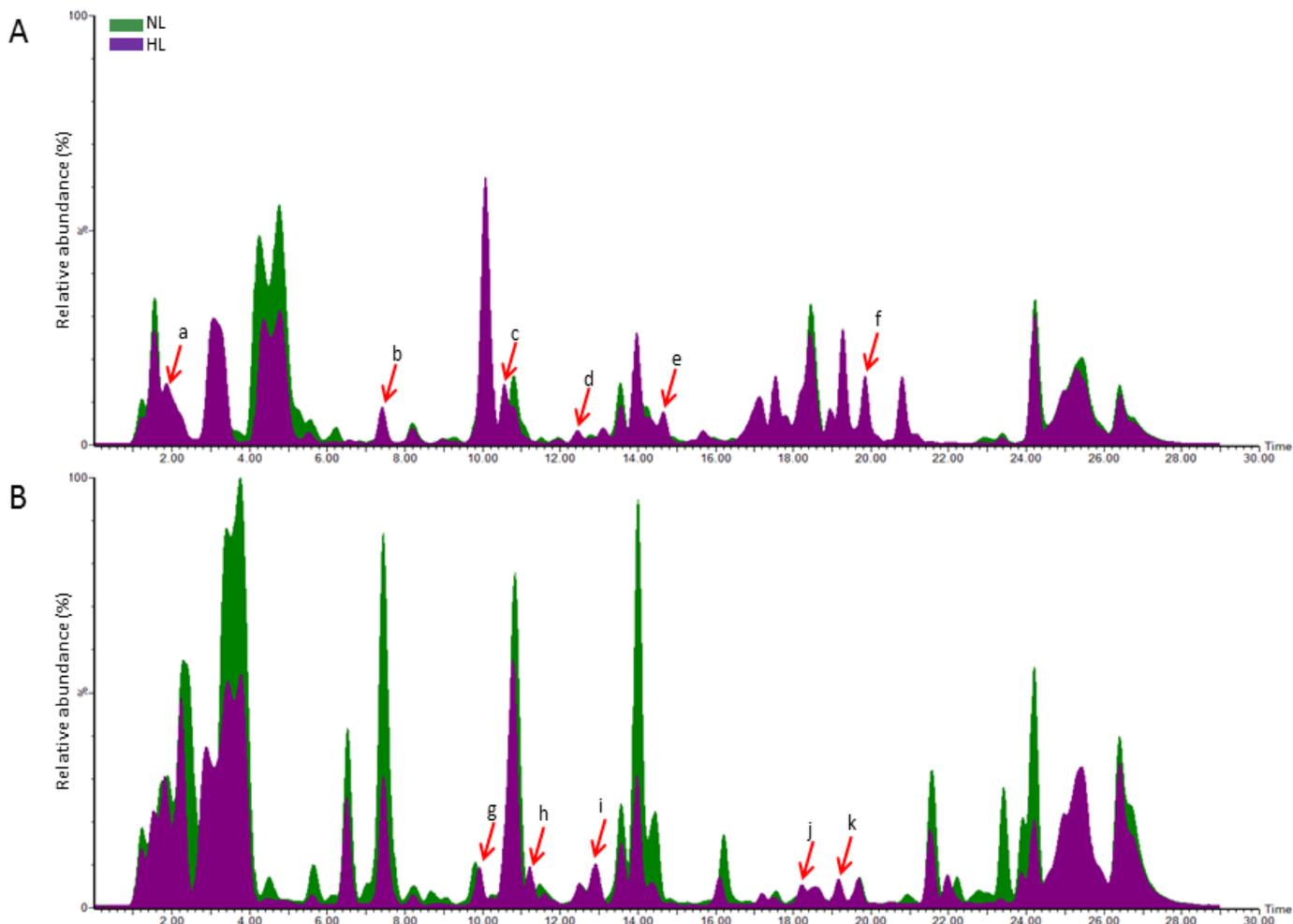
Figure 6: LC-MS/MS data indicating metabolic responses in (A) radish and (B) kale MGs grown under high light. The base peak ion chromatograms were extracted from each sample and overlaid to identify peaks (indicated by red arrows) that were present in HL (purple chromatogram) grown MGs, but absent in NL (green chromatogram) grown MGs. NL, normal light; HL, high light.

Table 1: Compounds in radish and kale MGs that accumulate as result of HL. Qualitative LC-MS/MS was conducted to predict which compounds accumulate in MG tissue when grown under HL conditions. The m/z spectrum was extracted from the respective peaks (refer to figure 6) and further used to confirm and/or predict known compounds accumulating as response to HL (a-k; using Metabolomics Workbench software).

Peak	Input mass (m/z)	Formula (neutral)	Exact mass (neutral)	Predicted Compound (s)	Compound class
a	341.1083	$C_{12}H_{22}O_{11}$	342.1162	● Galactinol ● Trehalose ● Mannobiose ● Kojibiose ● Sucrose ● Maltose	Carbohydrates

b	463.0487	$C_{16}H_{20}N_2O_{10}S_2$	464.0559	●	4-Hydroxy-3-indolylmethylglucosinolate	Glucosinolate
c	341.0878	$C_{15}H_{18}O_9$	342.0951	●●●	1-O-Caffeoylglucose; Caffeic acid 3-glucoside; Glucocaffeic acid	Glycoside
d	325.0915	$C_{15}H_{18}O_8$	326.1002	●	1-O-(4-coumaroyl)-beta-D-glucose; 2-(beta-D-glucosyloxy)-cis-cinnamic acid; 4-O-beta-D-glucosyl-4-coumaric acid; 4'-O-beta-D-glucosyl-cis-p-coumaric acid; Bilobalide; trans-beta-D-glucosyl-2-hydroxycinnamic; trans-o-Coumaric acid 2-glucoside	Terpene glucoside
e	591.1005 (fragment ion)	$C_{37}H_{30}O_{17}$	746.1483	●	Epigallocatechin-(4beta->8)-epicatechin-3-O-gallate ester	Proanthocyanidin
f	901.2430	$C_{42}H_{46}O_{22}$	902.2481	●	Kaempferol O-glucoside; Quercetin rhamnoside; Isovitexin glucoside; Variabiloside C and D	Flavonoid
g	353.0846	$C_{16}H_{18}O_9$	354.0951	●	5Z-Caffeoylquinic acid; Biflorin; Chlorogenic acid; Scopolin; Trans-5-O-caffeoyl-D-quinic acid; Trans-Chlorogenic acid; Cis-5-Caffeoylquinic acid	Phenolic acid
h	371.0964 (fragment ion)	$C_{23}H_{18}O_{11}$	470.0849	●	3,5,7-Tris(acetyloxy)-2-[4-(acetyloxy)-3-hydroxyphenyl]-4H-1-benzopyran-4-one	Flavonoid
i	628.1627	$C_{24}H_{43}N_3O_{16}$	629.2643	●	N-acetyl-D-galactosaminyl-(1->4)-(N-acetyl-D-galactosaminyl)-(1->3)-N-acetyl-D-galactosaminitol	Aminooligosaccharide
j	746.1956	$C_{34}H_{35}O_{19}$	747.1773	●	Cyanidin 3-(2G-galloylrutinoside); Cyanidin 3-O-(2"-O-galloyl-6"-O-alpha-rhamnopyranosyl-beta-galactopyranoside	Flavonoid
k	753.2256	$C_{25}H_{42}N_2O_{22}$	754.1950	●	alpha-Neup5Ac-(2->3)-beta-D-Galp-(1->3)-alpha-D-GalpNAc6S	Carbohydrate sulfate

DISCUSSION

Phytochemicals are associated with the health benefits of whole-plant based diets. A consumer market that seeks to include plants with enhanced phytochemical profiles has stimulated research efforts to (i) understand the metabolic fluxes during different developmental stages of plant growth (*e.g.* immature MG *vs.* mature), and (ii) develop effective bio-fortification strategies (using environmental stresses such as high light) to diversify phytochemical profiles. The recent interest and metabolite profiling of a range of MGs from Brassica species established their superior nutritional qualities over mature vegetables [20, 21, 28, 33]. Consequently, MGs have shifted from culinary garnish to health-promoting specialty crop. A major benefit of MG crops is the time it takes between planting and harvesting (approximately 10-25 days), resulting in a sustainable and steady supply of health beneficial plant-based foods rich in phytochemicals.

Although descriptive studies have inventoried the phytochemical profile of a range of MGs [27, 34], recent studies indicate a shift toward the understanding of how environmental bio-fortification strategies (light, temperature, water) could be used to diversify this profile [35, 36, 37]. During this early stage of development (MG), plants are exceptionally adaptable and respond rapidly to changes in their environment [38]. Exposure to HL is known to activate several stress-responsive pathways, including photosynthesis and redox signalling, resulting in altered carbon metabolism and energy balance [39, 40, 41, 42, 43]. Redox signalling causes increased levels of reactive oxygen species (ROS) in plant cells, which are toxic and can lead to severe cellular damage. A concomitant increase in phytochemicals is required as ROS scavengers (antioxidants) to protect the plant from oxidative damage [44, 45, 46]. In a previous study on wild rocket MGs, we demonstrated that HL bio-fortification resulted in the accumulation of resveratrol, a known ROS scavenger, and previously undocumented phytochemical in wild rocket [18]. Similarly, in this study, our objective was not to report on   descriptive phytochemical profiling   but rather to determine which phytochemicals uniquely appear in radish (root vegetable) and kale (leafy vegetable) MGs as a consequence of HL. By exploiting the increased ROS levels for the production of health beneficial phytochemicals (*in planta*), such bio-fortification techniques could be used in future to predictably diversify phytochemical profiles of MGs.

Phenotypic HL-induced responses include altered leaf and root morphogenesis as well as the inhibition of hypocotyl growth in both radish and kale MGs (Figure 1). Interestingly, radish initiated the development of a storage root, a phenomenon known as escape tropism, which allows roots to escape from unfavorable   light conditions. This is well-   known escape strategy used by mature plants and to our knowledge; this is the first report of such rapid production of storage roots in radish MGs. Furthermore, these radish 'micro-roots' displayed its typical red coloration. HL-exposed roots are associated with an increased phenylpropanoid metabolism [47], inducing the accumulation of flavonoids and anthocyanins (considered the most prominent color pigments in plants; Figure 6, Table 1). This type of phytochemical accumulation in the roots is important for stress adaptation, suggesting its role as a common intermediate in light signalling pathways to regulate root development [48, 49, 50].

Several flavonoids are mediated by PAP transcription factors [51, 52, 53]. PAP-mediated pathways require sucrose, an energy resource that plants produce *via* photosynthesis. An upregulation of flavonoid production could, therefore, result in increased carbohydrate levels   as a result of the careful regulation between sucrose and starch production during photosynthesis [54, 55, 56]. Increased total starch concentrations were observed for both radish and kale MGs

suggesting that the redistribution and/or accumulation of carbohydrates are important physiological adaptations to HL (Figure 2). Fold-change increases in radish MG carbohydrates (including sucrose) further supports the idea that redistribution occurs, complementing the rapid production of storage 'micro-roots' (Figure 6, Table 1). Interestingly in kale MGs, the amino oligosaccharide and carbohydrate sulfate compounds are known to be triggered as part of defense mechanisms, have also been upregulated [57, 58].

Such adaptations in the photosynthetic machinery have been well described and include redox signals arising from chloroplasts [59]. Ascorbic acid is known to accumulate in chloroplasts under HL conditions and   believed to function as (i) photosystem electron donors, (ii) ROS scavengers, and (iii) co-factors to produce non-photochemical quenching metabolites [60]. Most Brassica vegetables are naturally rich in ascorbic acid, and it was surprising that kale MGs did not accumulate significantly more ascorbic acid in response to HL. Surprisingly, radish MGs accumulated significantly less ascorbic acid in response to HL (Figure 3). The production of ascorbic acid for ROS scavenging purposes during MG stage might either be (i) too taxing on the plant system or (ii) fully converted to other small molecules in its capacity as co-factor. It would be interesting to further investigate the regulatory mechanisms involving photosynthesis and ascorbic acid production in these MGs under HL. We speculate that radish and kale MGs rely largely on the upregulated phenylpropanoid pathway, producing a diverse array of phytochemicals to reduce the damage from oxidative stress.

Despite the unexpected effect of HL on ascorbic acid, the accumulation of phytochemicals collectively resulted in increased total antioxidant capacity (TAC) in both radish and kale MGs (Figure 4). Increased ROS production is compensated for by the increased phytochemical production, which collectively functions as an antioxidant system [61]. Rather than a description of all accumulated phytochemicals, our study purposely focused on tentative identification of phytochemicals that differentially accumulated in response to HL (Figure 6, Table 1). In radish, the major groups of phytochemicals included glucosinolates, flavonoid glycosides, terpene glucosides, proanthocyanidins and flavonoids (Table 1). All these groups have been directly or indirectly implicated in plant responses to light stress [53, 61]. The only glucosinolate that accumulated in response to HL was 4-hydroxy-3-methyl glucosinolate. Oxidative stress studies, using methyl jasmonate, in Arabidopsis and pak choi (*Brassica rapa*) resulted in enhanced accumulation of 3-indolyl-methyl-glucosinolates [62, 63]. Interestingly, the use of salicylic acid (inhibitor of jasmonate signalling) indicated a differential upregulation of 4-hydroxy-3-methyl glucosinolate implicating the importance of this compound in stress response mechanisms of Brassica [62, 64]. Furthermore, the accumulation of a proanthocyanidin (epigallocatechin-(4beta->8)-epicatechin-3-O-gallate ester), commonly found in green tea, was also identified in radish MGs and is well described as a stress response compound in plants [1, 65]. In kale, the major groups of phytochemicals included flavonoids and phenolic acids, which include   organic and chlorogenic acids (CGAs). One of the most abundant polyphenols included in the human diet is CGA, which has several   important roles in the therapeutic properties of many plant extracts, such as antioxidant activity. Collectively, all these groups have, to some extent,   shown to confer antioxidant capacity (in both humans and plants), and some studies have demonstrated effectiveness in disease (*i.e.* cardiovascular, diabetes, obesity) prevention [2, 66, 67].

Despite the existence of several assays measuring antioxidant capacity in plants and plant extracts [68], the TAC kit used in this study (based on the copper-reducing antioxidant capacity

method or CUPRAC) has the ability to measure the non-enzymatic TAC of biological samples, indicating their ability to counteract oxidative stress-induced damage in cells. Validation of this TAC in MG extracts included the use of Fenton's reagent to demonstrate how extracts from the radish and kale MGs could protect DNA from the oxidative damage incurred by H_2O_2 (Figure 5). Extracts from both radish and kale MGs were equally effective at protecting DNA from oxidative damage at the highest concentration and exhibited no DNA protection at the lowest concentration, regardless of growing conditions. Comparatively, at concentrations of 100 ⌈g ml^{-1} radish MG extracts, grown under NL, exhibited the most effective DNA protection ability. The ascorbic acid concentrations were highest in radish MGs grown under NL and it is interesting to speculate that the concomitant DNA protection could be due to ascorbic acid. Ascorbic acid is a non-enzymatic, water soluble antioxidant obtained from the diet and essential to humans because of its role in several physiological activities [69]. The antioxidant activity of ascorbic acid has been demonstrated to prevent the development of certain types of cancer, cardiovascular diseases and other illnesses related to oxidative stress [70, 71]. Furthermore, ascorbic acid can convert reactive compounds to less toxic and more easily excreted products, as well as capturing free radicals before they interact with other molecules, protecting them from oxidative damage [72, 73]. However, many of these i*n vitro* and *in vivo* studies are inconclusive, and it is suggested that compounds work synergistically (as a holistic antioxidant system).

In conclusion, the purpose of this study was to investigate the effects of HL on phytochemical accumulation and TAC in radish and kale MGs. Humans rely on a diverse array of vitamins, minerals, and phytochemicals for general and preventative health purposes, the latter of which are obtained solely from plant-based foods. Functional plant-based foods and food products contain abundant amounts of health-beneficial phytochemicals, which provides enhanced antioxidant capacity to prevent diseases associated with oxidative damage. MGs are known to contain up to 100 times more phytochemicals than mature plants, and it is proposed as an effective health beneficial speciality crop in the current global state of malnutrition, climate variability, and the availability of arable land. Such nutritious and phytochemical-rich speciality crops could be farmed (and bio-fortified simultaneously) vertically in controlled conditions, reducing the time from farm-to-table. Further enhancement and diversification of MG profiles through HL bio-fortification will ensure not only a resourceful fresh-food product but also a strategy for making dried supplemental products which could be used to fortify other food sources with a longer shelf life (such as bread, maize meal) as a means to human health and nutrition.

List of abbreviations
CGA – chlorogenic acid
CUPRAC – cupric reducing antioxidant capacity
DW – dry weight
HL – high light
LC-MS/MS – liquid chromatography mass spectrometry
MG – micro-greens
NL – normal light
ROS – reactive oxygen species
TAC – total antioxidant capacity

Competing interests: None

Author contributions
AX and conducted metabolite extractions, ascorbic acid and TAC assays, and conducted DNA damage/protection assays. PM assisted with DNA damage assays as part of a learner programme. MS executed LC-MS/MS experiments and EH analysed LC-MS/MS data. NK executed starch assays. BL conceived of the project, interpreted the work and wrote the manuscript. SP actively provided valuable intellectual input and assisted in the writing of manuscript. JK provided infrastructure and funding toward the project.

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