







Evaluation of growth and nutritional value of Brassica microgreens grown under red, blue and green LEDs combinations

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Microgreens are rich functional crops with valuable nutritional elements that have health benefits when used as food supplements. Growth characterization, nutritional composition profile of 21 varieties representing five species of the *Brassica* genus as microgreens were assessed under light-emitting diodes (LEDs) conditions. Microgreens were grown under four different LEDs ratios (%); red:blue 80:20 and 20:80 (R₈₀:B₂₀ and R₂₀:B₈₀), or red:green:blue 70:10:20 and 20:10:70 (R₇₀:G₁₀:B₂₀ and R₂₀:G₁₀:B₇₀). Results indicated that supplemental lighting with green LEDs (R₇₀:G₁₀:B₂₀) enhanced vegetative growth and morphology, while blue LEDs (R₂₀:B₈₀) increased the mineral and vitamin contents. Interestingly, by linking the nutritional content with the growth yield to define the optimal LEDs setup, we found that the best lighting to promote the microgreen growth was the green LEDs combination (R₇₀:G₁₀:B₂₀). Remarkably, under the green LEDs combination (R₇₀:G₁₀:B₂₀) conditions, the microgreens of Kohlrabi purple, Cabbage red, Broccoli, Kale Tucsan, Komatsuna red, Tatsoi and Cabbage green, which can benefit human health in conditions with limited food, had the highest growth and nutritional content.

Introduction

As the world's population is rapidly growing, with an increasing demand for sustainable sources of food

products such as the rich-nutrient functional crops, ongoing efforts are aimed to find new strategies for food production to meet the increasing demands. Recently, the

Abbreviations – DAD, photodiode array detector; DW%, dry weight percentage; FW, fresh weight; GA, gibberellins; HL, hypocotyl length; HPLC, high performance liquid chromatography; ICP-OES, inductively coupled plasma optical emission spectrometry; LA, leaf area; LEDs, light-emitting diodes; LOD, limit of detection; LOQ, limit of quantification; PPFD, photosynthetic photon flux density; RH, relative humidity; RP-HPLC, reverse-phase high performance liquid chromatography; TAA, total ascorbic acid.

consumption of microgreens has increased due to its high level of nutritional components, such as vitamins, minerals and antioxidants compared to mature greens, which are helpful in filling the nutritional gap challenges (Burlingame 2014). Furthermore, microgreens are valuable functional crops because of their rich-phytonutrients content (Kyriacou et al. 2016, Bulgari et al. 2017). Microgreens are harvested at the base of the hypocotyl, when the first true leaves start to emerge with about 3 weeks after sowing (Treadwell et al. 2010, Xiao et al. 2012). Despite their small size, they can provide a high concentration of health-promoting phytochemicals (Xiao et al. 2012). Commercially greenhouse growers became interested in the microgreen for their high market values (Treadwell et al. 2010). Specifically, microgreens of the family *Brassicaceae* have become a popular choice due to its easy germination, short growing time, variety of flavors and vibrant colors (Xiao et al. 2012). *Brassicaceae* microgreens species could be used as a new ingredient that provides a wide variety of novel foods (Lee et al. 2004, Murphy and Pill 2010, Xiao et al. 2012), and for its health value due to the high amounts of cancer-fighting glucosinolates (Kopsell and Sams 2013). They are also rich in carotenoids, especially lutein, zeaxanthin and β -carotene (Lefsrud et al. 2007, Björkman et al. 2011, Carvalho and Folta 2014). Thus, *Brassica* microgreens are a functional food which can be used as a supplement to promote health or prevent disease (Xiao et al. 2012, Kou et al. 2013). Plant breeding has caused this group of crops to be highly variable in appearance, phytochemicals and uses (Lefsrud et al. 2007, Björkman et al. 2011, Carvalho and Folta 2014), have with health-promoting or disease preventing properties (Xiao et al. 2012, Kou et al. 2013). Carotenoids are characterized by such functions as free-radical scavenging, enhancing the immune response, suppressing cancer development and protecting eye tissues, but individual carotenoids differ in their protective roles. α -carotene, β -carotene and cryptoxanthins, which are pro-vitamin A carotenoids, are mostly associated with cardiovascular disease reduction. Zeaxanthin and lutein are components of the macular pigment in the eye and protect the macula from light-induced damage. Lycopene prevents cardiovascular diseases and prostate cancer (Botella-Pavía and Rodríguez-Concepción 2006, Kopsell and Kopsell 2006).

Several strategies have been developed for improving the yield of microgreen by optimizing the growth conditions. Light-emitting diode (LED) lighting is a new light source technology for plant growth chambers (Yorio et al. 2001, Yano and Fujiwara 2012). By customizing light qualities and intensities with LEDs, efficiency is improved and costs are reduced. This makes LEDs an economically viable alternative for indoor farming

(Goto 2012). Plants responded to the different light intensity and wavelength (Samuolienė et al. 2013a, Dong et al. 2014). Microgreens have a lower demand for photon flux compared to long-cycle crops, thus are ideally adapted to chamber environments. Recently, many studies have demonstrated the influence of LEDs (blue and/or red) lighting on the plant vegetative parameters (Yorio et al. 2001, Matsuda et al. 2004, Matsuda et al. 2008) and demonstrated the effect of light quality on the growth of the cultivated plants (Stutte et al. 2009, Tarakanov et al. 2012, Kopsell and Sams 2013, Samuolienė et al. 2013b). Nevertheless, there is a lack of information regarding the combined effect of red and blue and other LEDs lighting such as green light on the plant growth, morphology and nutrition content of microgreens (Samuolienė et al. 2013b, Ouzounis et al. 2015).

Although microgreens have been considered as valuable and nutritionally beneficial functional crops, little is known regarding the integrity of individual and combined influence of green, red and blue LEDs on *Brassica* species microgreens growth and nutritional composition. Therefore, the main purposes of the current study are to define the influence of alternative LEDs light regimens on *Brassica* species microgreens growth, and nutritional composition, and to define which species could serve well as a life support component. We explored the impact of different LEDs lighting ratios (red, blue and green) on the overall growth and nutritional profile of 21 *Brassica* microgreens.

Material and methods

Plant materials and growth chamber environment

Twenty-one varieties of microgreens representing five species of *Brassica* genus of the *Brassicaceae* family (Table 1) were grown in controlled-growth chambers in a collaborative study between the Faculty of Agriculture in Zagazig University and Cairo University, Egypt. We used the recommended soil and fertilization properties (Xiao et al. 2012). About 10–25 g of seeds, varying based on the seed index of each variety (Table 1) were sown in peat moss in Rockwool tray in a controlled conditions growth chambers (three trays per each variety for three replicates), cultivated under relative humidity (RH), and carbon dioxide (CO₂) concentration of 70%, and 500 $\mu\text{mol mol}^{-1}$, respectively. Each day, 100 ml of 0.1 M CaCl₂ solution was added to each tray to further stimulate seedling growth. Once cotyledons were fully reflexed 5 d after sowing, 300 ml of 25% nutrient solution was added to each tray daily until harvest. This experiment was carried out simultaneously for three replicates with a growth duration [period from sowing until harvest] for each species ranging from 6 to 12 days (Table 1).

Table 1. Twenty-one varieties of *Brassica* microgreens representing five species *Brassica* genera assayed in this study. Growth duration (day) and seed index (g) show each variety growth period and the number of seeds used for the sowing.

Commercial name	Scientific name (genus and species)	Growth duration (day)	Seed index (g)
Broccoli	<i>Brassica oleracea</i> L. var. <i>italica</i>	9	10
Brussel sprouts	<i>B. oleracea</i> L. var. <i>Gemmifera</i>	10	15
Cabbage green	<i>B. oleracea</i> L. var. <i>capitata f. alba</i>	9	10
Cabbage red	<i>B. oleracea</i> L. var. <i>capitata f. rubra</i>	8	10
Cabbage savoy	<i>B. oleracea</i> L. var. <i>capitata f. sabauda</i>	8	10
Cauliflower	<i>B. oleracea</i> L. var. <i>botrytis</i>	9	15
Collard	<i>B. oleracea</i> L. var. <i>viridis</i>	10	15
Kale	<i>B. oleracea</i> L. var. <i>alboglabra</i>	10	15
Chinese Kale red	<i>B. oleracea</i> L. var. <i>acephala</i>	9	10
Kale Tucsan	<i>B. oleracea</i> L. var. <i>acephala</i>	9	15
Kohlrabi purple	<i>B. oleracea</i> L. var. <i>gongylodes</i>	7	25
Cabbage Chinese	<i>Brassica rapa</i> L. var. <i>pekinensis</i>	6	15
Komatsuna red	<i>B. rapa</i> L. var. <i>perviridis</i>	8	15
Mizuna	<i>B. rapa</i> L. var. <i>nipposinica</i>	8	15
Pak choy	<i>B. rapa</i> L. var. <i>chinensis</i>	8	15
Rapini	<i>B. rapa</i> L. var. <i>ruvo</i>	9	15
Turnip	<i>B. rapa</i> L. var. <i>rapa</i>	9	10
Mustard Dijon	<i>Brassica juncea</i> (L.) Czern.	12	15
Mustard red	<i>B. juncea</i> (L.) Czern.	10	10
Rutabaga	<i>Brassica napus</i> L. var. <i>napobrassica</i>	9	10
Tatsoi	<i>Brassica narinosa</i> L. var. <i>rosularis</i>	7	10

LEDs lighting treatments

Brassica microgreen plants were grown under LEDs (LED arrays) lighting provided by four different light quality ratios (%) treatments of red:blue 80:20, and 20:80 (R₈₀:B₂₀, and R₂₀:B₈₀), or red:green:blue 70:10:20 and 20:10:70 (R₇₀:G₁₀:B₂₀ and R₂₀:G₁₀:B₇₀) (Philips Green-Power LED production modules; Koninklijke Philips Electronics, The Netherlands), using 0.5 W per LED chip. In the controlled environment growth chamber, the LEDs were placed horizontally, above the benchtop, at a height of 50 cm. We adjusted the photosynthetic photon flux density (PPFD) average to 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ that was provided by the fluorescent lamps and bar-type LEDs.

The experiment was performed with three times replications in the same conditions.

Harvest and growth measurements

Microgreen samples were harvested after the growth duration for each species (Table 1) without seed coats or roots as recommended (Xiao et al. 2012). Each replicate used for the measurements consisted of at least 10 seedlings. Ten seedlings of each microgreen variety were randomly selected and measured to determine hypocotyl length (HL), leaf area (LA), for each LEDs treatment. Hypocotyl (HL) measurements of the harvested seedlings were measured from the tip where the cotyledons split, to the end of the base of the hypocotyl. Leaf Area of cotyledons using fully expanded leaves were measured by LA meter (LI-3100; LI-COR Inc.) by recording the average of five scans. Furthermore, another 10 randomly selected seedlings for each variety used to assess both, fresh weight (FW) and dry weight percentage (DW%). After FW data were measured, samples were oven-dried at 80°C for 72 h, then DW data were measured. FW and DW values were used to calculate DW% [DW% = (DW/FW × 100)].

Elemental analysis

Fresh microgreens (50 g FW per each sample) were collected and rinsed 3 times using H₂O to remove any surface residue. Dried microgreens (2 g per replicate) were grounded into a fine powder to analyze the elemental composition. Each of the 21 samples was subjected to acid digestion procedures and quantitative measurements of P, K, Ca, Na, Fe, Mn, Cu and Zn using inductively coupled plasma optical emission spectrometry (ICP-OES) following the methods of Huang and Schulte (1985). To assure the accuracy of the method, standard reference materials (Apple leaves, NIST[®] SRM[®] 1515, NIST1515, SIGMA, USA and Spinach leaves, NIST[®] SRM[®] 1570a, NIST1570A) were used and evaluated using the same lysis procedure. For each ICP-OES analyte, the limit of detection (LOD) and limit of quantification (LOQ), which are a function of the sample mass were determined (Table S1, Supporting Information).

Vitamin and carotenoid concentrations

Phylloquinone

Phylloquinone (vitamin K₁) was determined according to a previously reported method (Booth et al. 1994). Under dime light, 0.2 g of dried microgreens were homogenized in 10 ml of H₂O and 0.4 ml of 200 $\mu\text{g ml}^{-1}$ menaquinone used as an internal standard. The sample was supplied with 15 ml of 2-propanol/hexane (3:2, v/v) and vortexed for 1 min. The sample was centrifuged at 1500 g at

21°C for 5 min. The upper layer (hexane) was transferred into a new glass tube and dried using a stream of N₂. The residues of the sample were dissolved using 4 ml of hexane. To purify the extract, 1 ml of the dissolved extract was loaded onto preconditioned silica gel columns (4 ml of 3.5% ethyl ether in hexane, followed by 4 ml of 100% hexane). Two milliliters of hexane was used to wash the columns. Phylloquinone was eluted with 8 ml of 3.5% ethyl ether in hexane and then evaporated at 40°C under N₂ flow. Further, it was reconstituted in 2 ml of mobile phase (99% methanol and 1% 0.05 M sodium acetate buffer, pH = 3.0) and filtered through a 0.22 µm nylon syringe filter (Millipore, Bedford, MA). To detect the phylloquinone, we used a photodiode array detector (DAD) (G1315C, Agilent, Santa Clara, CA) on Agilent 1200 series HPLC system, wherein the absorbance wavelength was 270 nm. Twenty microliters of the extract was injected into the HPLC and being run through a C18 column (201TP, 5 µm, 150 × 4.6 mm, Grace, Deerfield, IL) flowing at the rate of 1 ml min⁻¹. The phylloquinone content was measured according to the internal standard method based on peak areas.

Ascorbic acid

Total ascorbic acid (TAA) (vitamin C) was assessed spectrophotometrically (Hodges et al. 2001). Three grams of fresh microgreens were homogenized in 10 ml of ice-cold 5% metaphosphoric acid (w/v) at 2515 g for 1 min at 4°C. The homogenized centrifuged at 7000 g for 20 min at 4°C. The supernatant was filtered through Whatman 4 filter paper. TAA was measured spectrophotometrically at 525 nm. Concentrations of TAA were calculated from L-ascorbic acid standard curve.

Carotenoids and tocopherols

To extract both carotenoids (provitamin A) and tocopherols (vitamin E), we followed the procedure described (Lester et al. 2010) and modified by Xiao et al. (2012). In 15 ml screw-cap glass vial, 0.05 g of dried fine powder was homogenized in 7.5 ml of 1% butylated hydroxytoluene in ethanol and 500 µl of 86.82 µM trans-βapo-8 carotenal as an internal standard was added. A total of 180 µl of 80% KOH was supplied to the mixture and, the vials were capped and placed in a dry bath at 70°C for 15 min. The vials were removed and being cooled on ice 4°C for 5 min. The mixture was transferred into 15 ml centrifuge tubes supplied with 3.0 ml of deionized water, and 3.0 ml of hexane/toluene solution (10:8, v/v). The mixture was carefully vortexed for 1 min then centrifuged at 1000 g for 5 min. After centrifugation, the upper organic layer was collected into an 8 ml glass culture tube and immediately placed into a nitrogen evaporator

set at 30°C. On the other hand, the lower layer was extracted with 3.0 ml of hexane/toluene (10:8, v/v). This extraction process was repeated at least four times until the upper layer is colorless. After evaporation, the residue was diluted in 500 µl of mobile phase acetonitrile/ethanol (1:1, v/v), filtered into an HPLC amber vial using nylon filter (0.22 µm, Millipore). Subsequently, 20 µl was inoculated for HPLC analysis. Carotenoid and tocopherol concentrations were measured using isocratic reverse-phase high performance liquid chromatography (RP-HPLC). Absorbance was measured at 290 nm (for tocopherols) and 450 nm (for carotenoids).

Clustering hierarchical analysis

In order to extrapolate the similarities and the dissimilarities among the 21 microgreens in growth and nutritional assessment, hierarchical cluster analysis was performed using the normalized data set using class Orange clustering hierarchical using ORANGE version 3.7 (Demsar et al. 2013).

Statistical analysis

The experiment was laid out in a randomized block design in a factorial arrangement with LEDs (four levels) and Microgreens (Twenty-one varieties) for three different biological replicates. Data were collected and analyzed (Steel and Torrie 1980). SPSS_{v.22} software was used to analyze the variance of differences using ANOVA test statistically followed by LSD analysis. The degree of freedom was followed as $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ considers the statistical significance and represents as *, **, ***, respectively.

Results

The influence of LEDs on microgreens growth and nutritional profile

In the present work, four different LEDs lighting ratio (%) treatments of R₈₀:B₂₀, R₂₀:B₈₀, R₇₀:G₁₀:B₂₀ and R₂₀:G₁₀:B₇₀ were implemented. Growth parameters of the 21 varieties were analyzed (Fig. 1). Results revealed that those microgreens that were grown under the R₇₀:G₁₀:B₂₀ had the highest growth and morphology targeted parameter, while the lowest growth parameters were observed under R₂₀:B₈₀ (Fig. 1). The HL and LA of the microgreens were significantly elongated in plants grown under R₇₀:G₁₀:B₂₀ compared to those grown under R₈₀:B₂₀, R₂₀:B₈₀ and R₂₀:G₁₀:B₇₀, respectively (Fig.1). FW and DW% of microgreens grown under R₇₀:G₁₀:B₂₀ treatment showed the highest values; on average; 0.4 g (FW), and 6.27% (DW%), indicating that the R₇₀:G₁₀:B₂₀ combination induces an increase in all growth and morphology

parameters studied in comparison to the other LEDs lighting treatments (Fig. 1).

Considering that R₇₀:G₁₀:B₂₀ LEDs lighting combination has an impact on targeted growth parameters, we investigated whether it has a functional influence on the nutritional composition profile by conducting an ICP

analysis of macro- and microelements from 21 varieties *Brassicaceae* microgreen using the lowest growth enhancer combination as internal references. Unexpectedly, relative macro- and microelements content showed a dramatic decrease for R₇₀:G₁₀:B₂₀ LEDs lighting combination compared to R₂₀:B₈₀ and the other LEDs ratios

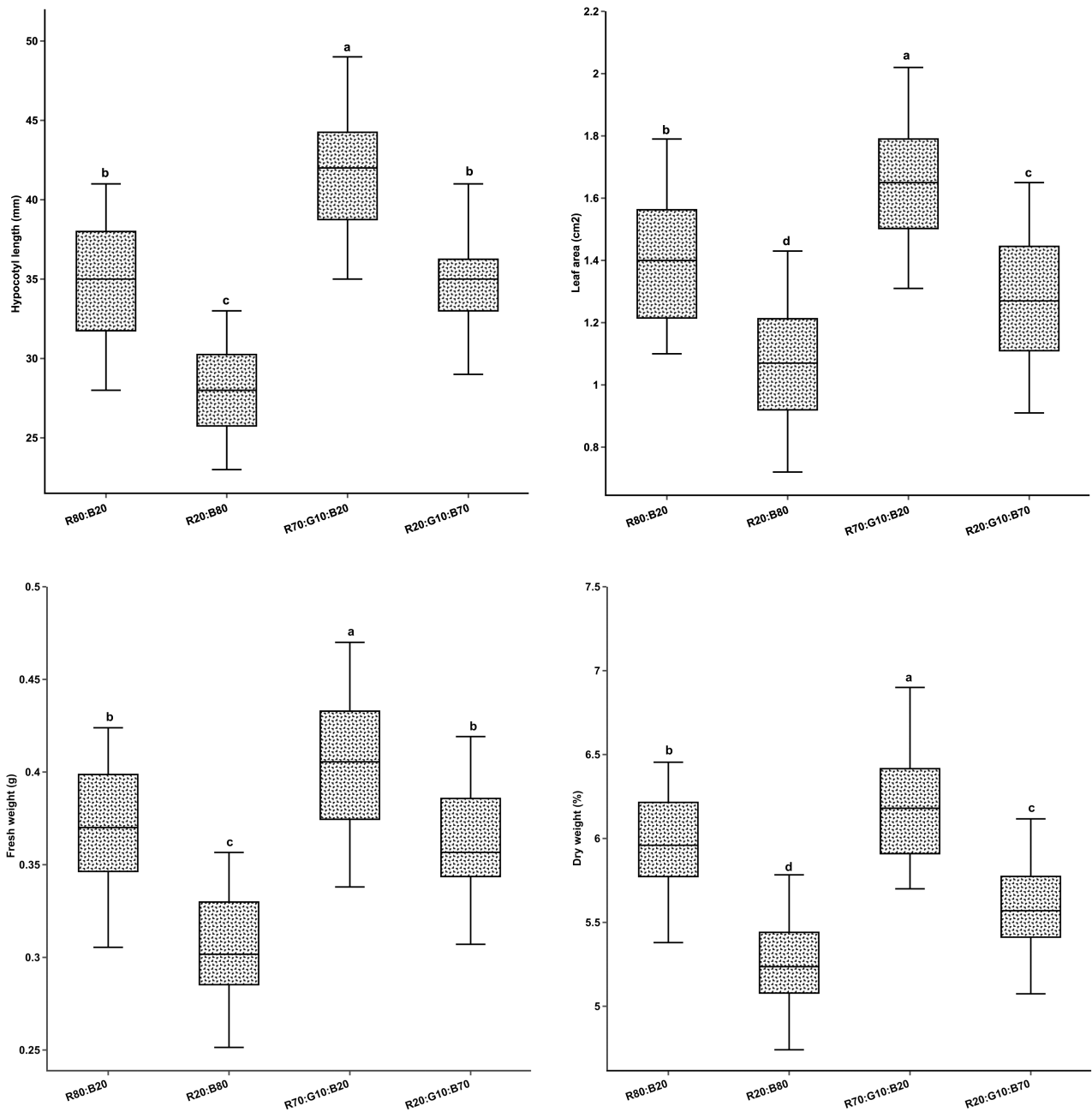


Fig. 1. Box plot of growth and morphological measurements of *Brassica* microgreens grown under LEDs treatments. The plot illustrates the mean and median of [hypocotyl length (mm), leaf area (cm²), FW (g) and DW (%)] of 21 varieties of *Brassica* microgreens representing five species grown under different LEDs ratio (%) of red:blue 80:20 (R₈₀:B₂₀), red:blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀) (Tables S2 and S3). Resulting ranking could be analyzed with point values of mean and median or uncertainty range with box. Statistical analysis is performed using a one-way ANOVA test ($P \leq 0.05$). Small letters denote statistically significant differences.

(Figs 2 and 3). Whereas the highest levels of macro- and microelements content were obtained in microgreens grown under R₂₀:B₈₀ combination. However, the analysis also did not take the yield into consideration.

Considering the influence of LEDs lighting combination on the microgreen's growth together with nutrition components value, we analyzed deeper the vitamin

and carotenoid contents. Agreeing with our previous result obtained in the macro- and microelements, we found that the contents of phyloquinone, α -tocopherol, TAA and β -carotene of 21 varieties of *Brassica* microgreens grown under red:blue 80:20 (R₈₀:B₂₀), were significantly increased compared to other combination (Fig. 4).

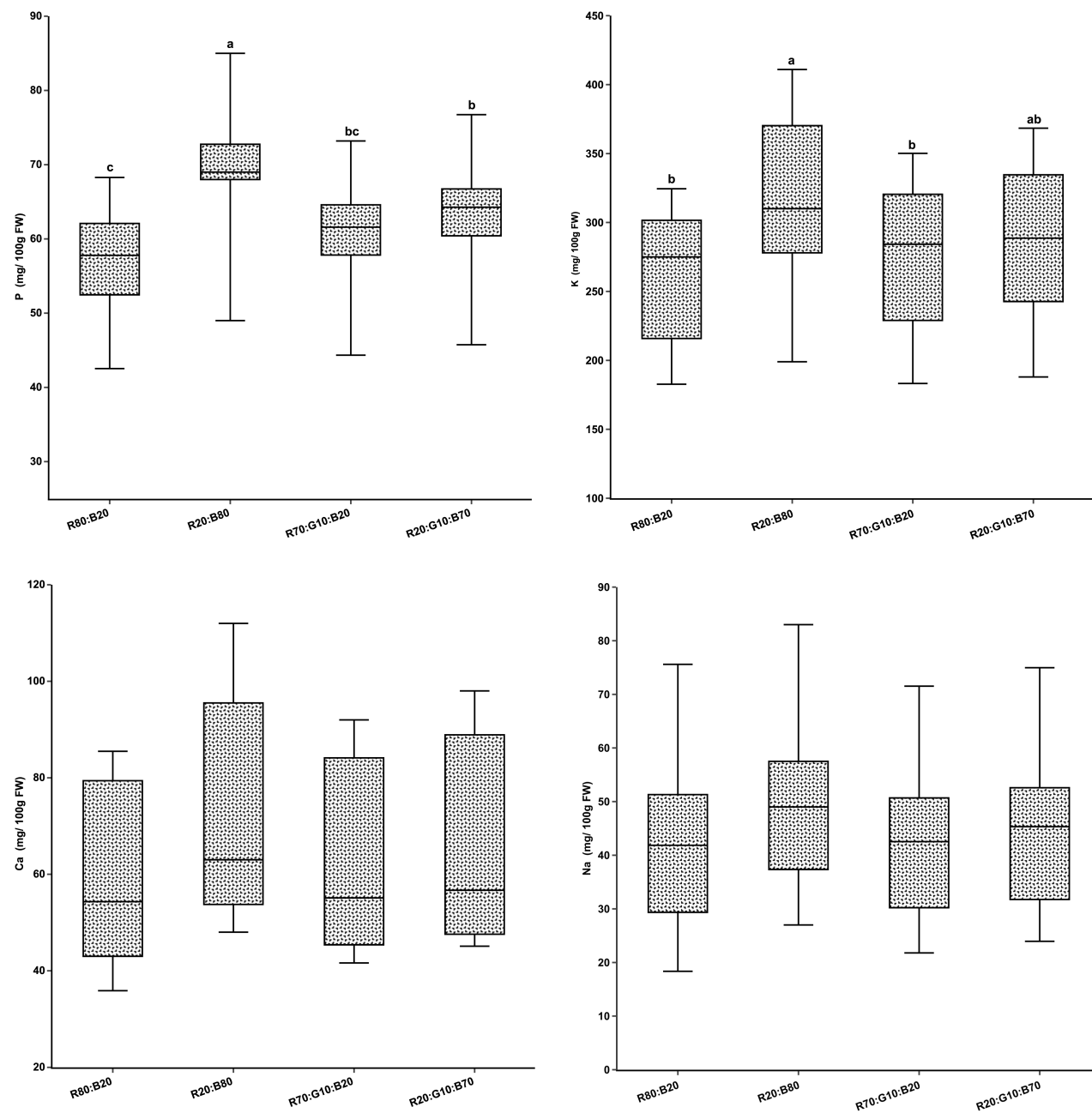


Fig. 2. Box plot of mineral composition and content of macroelements of *Brassica* microgreens grown under LEDs treatments. The plot illustrates the mean and median of macroelements concentrations; P, K, Ca and Na (mg/100 g FW) of 21 varieties of *Brassica* microgreens representing five species grown under different LEDs ratio (%) of red:blue 80:20 (R₈₀:B₂₀), red:blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀) (Tables S4 and S5). Resulting ranking could be analyzed with point values of mean and median or uncertainty range with box. Statistical analysis is performed using a one-way ANOVA test ($P \leq 0.05$). Small letters denote statistically significant differences.

Concluding the optimum LEDs conditions for *Brassica* microgreens growth conditions

Our previous data showed that LEDs lighting combination has an impact on all growth and nutritional parameters. More precisely, we found that *Brassica* microgreen varieties grown under the LEDs lighting of R₇₀:G₁₀:B₂₀

combination enhances the HL, LA, FW and DW% compared to other LEDs combinations, while minerals (macro- and microelements) and vitamins were significantly increased in plants grown under R₈₀:B₂₀. To detect the best LEDs combination, taking into consideration the actual yield of microgreens, we conducted a correlation

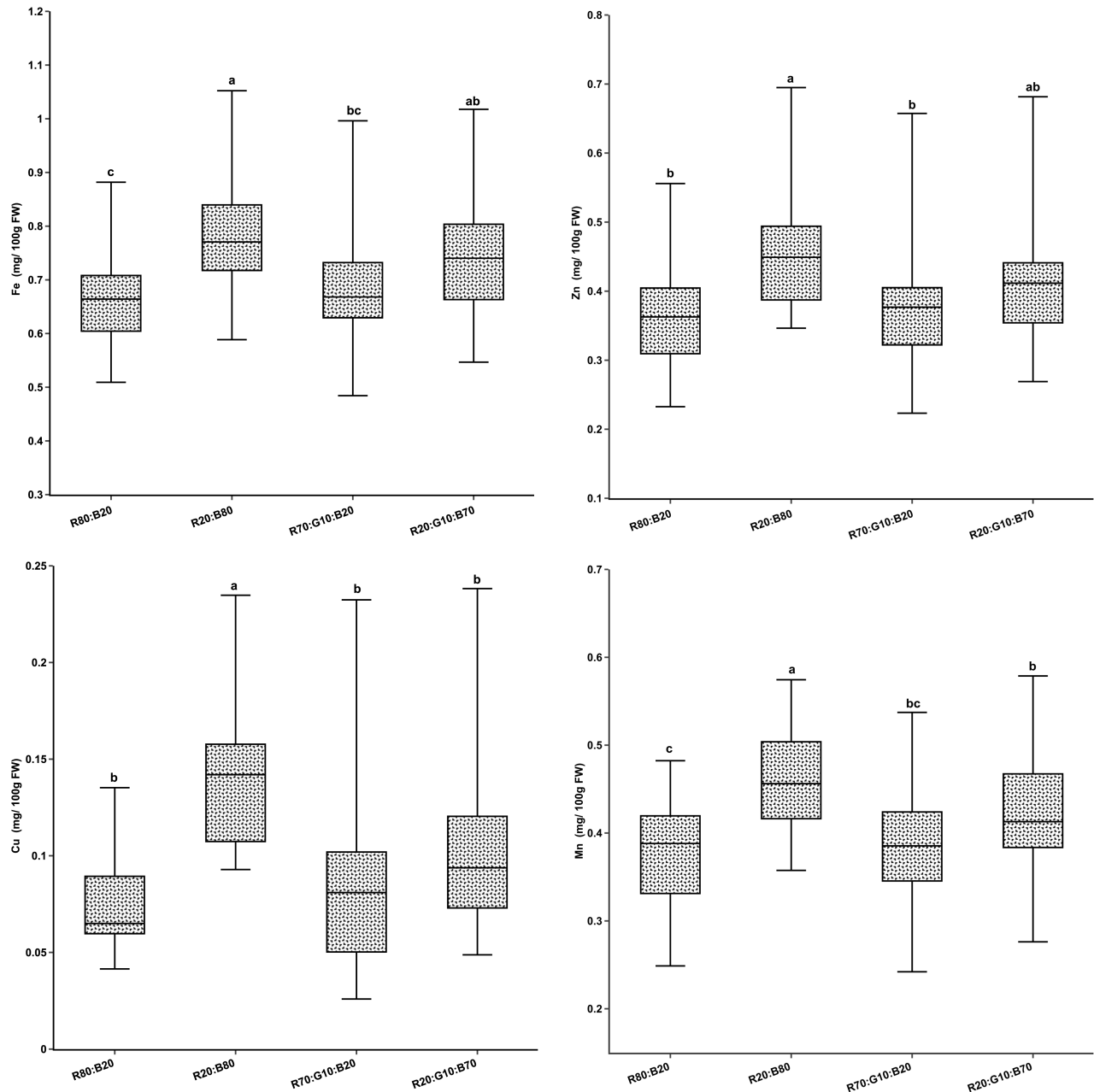


Fig. 3. Box plot of mineral composition and content of microelements of *Brassica* microgreens grown under LEDs treatments. The plot illustrates the mean and median of microelements concentrations; Fe, Zn, Cu and Mn (mg/100 g FW) of 21 varieties of *Brassica* microgreens representing five species grown under different LEDs ratio (%) of red:blue 80:20 (R₈₀:B₂₀), red:blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀) (Tables S6 and S7). Resulting ranking could be analyzed with point values of mean and median or uncertainty range with box. Statistical analysis is performed using a one-way ANOVA test ($P \leq 0.05$). Small letters denote statistically significant differences.

analysis with the yield. We estimated the minerals and vitamin concentrations corresponding to the actual FW yielded (Figs 5 and 6). Interestingly, we found that the mineral compositions and vitamins content in the

yielded FW were significantly increased in the micro-green varieties grown under the LEDs lighting of R₇₀:G₁₀:B₂₀ combination compared to other combinations (Figs 5 and 6).

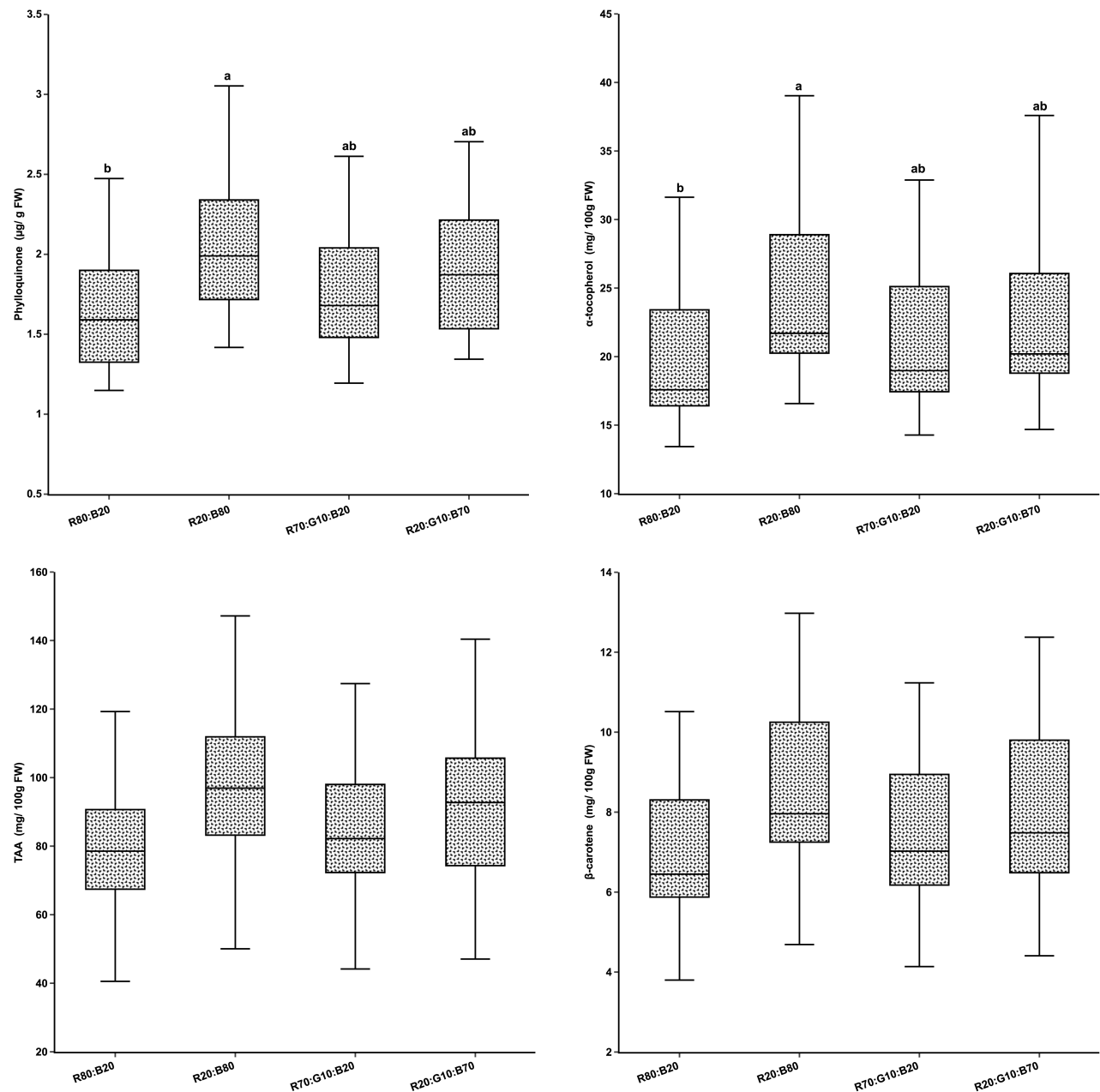


Fig. 4. Box plot of vitamin and carotenoid concentrations of *Brassica* microgreens grown under LEDs treatments. The plot illustrates the mean and median of vitamin and carotenoids concentrations; phyloquinone ($\mu\text{g g}^{-1}$ FW), α -tocopherol, TAA and β -carotene (mg/100 g FW) of 21 varieties of *Brassica* microgreens representing five species grown under different LEDs ratio (%) of red:blue 80:20 (R₈₀:B₂₀), red:blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀) (Tables S8 and S9). Resulting ranking could be analyzed with point values of mean and median or uncertainty range with box. Statistical analysis is performed using a one-way ANOVA test ($P \leq 0.05$). Small letters denote statistically significant differences.

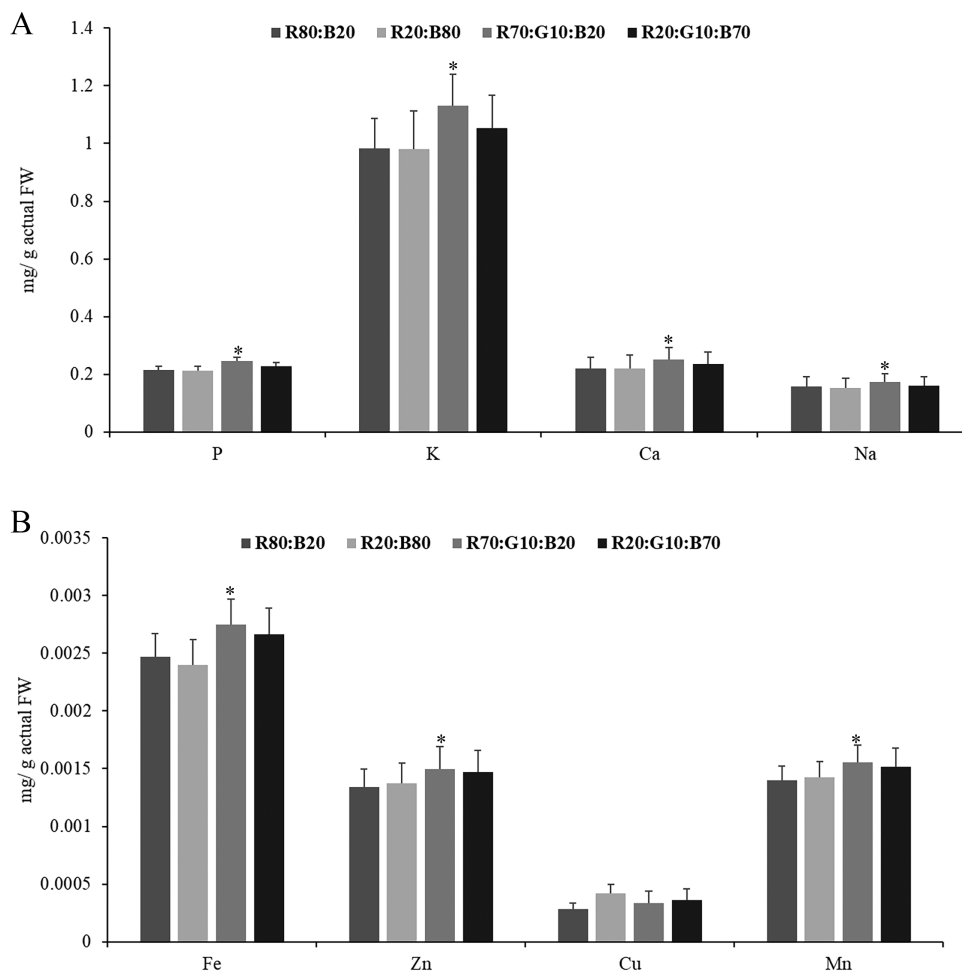


Fig. 5. Mineral composition and content of *Brassica* microgreens under LEDs treatments. (a) Mean macroelement concentration of P, K, Ca and Na. (b) Mean microelement concentration of Fe, Zn, Cu and Mn of 21 varieties of *Brassica* microgreens exposed to different LEDs ratio (%) of red:blue 80:20 (R₈₀:B₂₀), red:blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Data represents as a mean concentration corresponding to the actual FW (FW results of each LEDs treatments of the 21 varieties; Table 3) [actual concentration (mgg⁻¹ actual FW) = concentration (mg/100 g FW) × FW (g)/100]. Mean ± SE values are based on a representative sample from each treatment across three experimental replications. **P* ≤ 0.05.

Hierarchical cluster analysis of 21 varieties of *Brassica* microgreens

A hierarchical cluster analysis profiled growth, mineral compositions and vitamin content of 21 microgreens varieties grown under R₇₀:G₁₀:B₂₀ family has been performed using class Orange clustering hierarchical using ORANGE version 3.7 (Demsar et al. 2013). Data of microgreens grown under R₇₀:G₁₀:B₂₀, which presents the highest values of growth and nutritional profile are shown in Fig. 7 and Table 2. The hierarchical analysis methods (average-linkage distance between two clusters) were utilized to evaluate whether these trends were consistent across the 21 varieties. The hierarchical cluster analysis shows that the 21 microgreens are classified into

five groups. Among the five groups, the highest distance group (Fig. 7, cluster group in yellow color + Kale Tucsan in green cluster) contained seven microgreens (Kohlrabi purple, Cabbage red, Broccoli, Kale Tucsan, Komatsuna red, Tatsoi and Cabbage green) which are representing three species (*B. oleracea*, *B. rapa* and *B. narinosa*).

Discussion

Due to the growing interest to equip the growing chambers with LED lighting and for increasing the microgreen growth and nutritional profile, we investigated the impact of four different LEDs lighting ratios on the growth and nutritional quality of 21 varieties of the *Brassica* genera of the *Brassicaceae* family grown as microgreens.

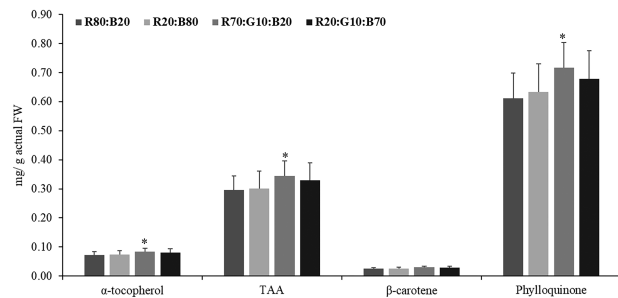


Fig. 6. The assessment of vitamin and carotenoid concentrations of *Brassica* microgreens under LEDs treatments. (A) Mean α -tocopherol, TAA and β -carotene (mg/100 g FW) concentration. (B) Mean phylloquinone ($\mu\text{g g}^{-1}$ FW) concentration of 21 varieties of *Brassica* microgreens exposed to different LEDs ratio (%) of red:blue 80:20 ($R_{80}:B_{20}$), red:blue 20:80 ($R_{20}:B_{80}$), red:green:blue 70:10:20 ($R_{70}:G_{10}:B_{20}$) or red:green:blue 20:10:20 ($R_{20}:G_{10}:B_{70}$). Data represent as a mean concentration corresponding to the actual FW (FW results of each LEDs treatments of the 21 varieties (Table S3) [actual concentration (mg g^{-1} actual FW) = concentration (mg/100 g FW) \times FW (g)/100]. For phylloquinone [actual concentration (mg/g actual FW) = concentration ($\mu\text{g g}^{-1}$ FW) \times FW (g)]. Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. * $P \leq 0.05$.

Microgreens are reported in many studies as valuable source of vitamins, phenolics and minerals (Mir et al. 2017). Enhancing their nutritional qualities and growth is an exciting avenue of research in agriculture.

In our study, we revealed various effects of the combination ratios between blue LEDs, red and green LEDs.

Growing plants under a monochromatic light beam stimulates specific photoreceptors that are involved in numerous biological processes. Enhancement the nutritional profile and plant growth has been demonstrated in many species, such as rice (Chen et al. 2014), *Brassica* spp. (Xiao et al. 2012, Samuolienė et al. 2013a, Samuolienė et al. 2013b). It has been reported that red and blue light are important for the expansion of hypocotyl elongation, pigment accumulation and enhancement of biomass (Li et al. 2010). It is reported that exposure to green LEDs increases biomass at high intensities (Johkan et al. 2010). We noticed that growing microgreens under $R_{70}:G_{10}:B_{20}$ gave the highest value of the vegetative parameters, taking in consideration the yield produced under all combination treatments (Fig. 1). These results provide a clear indication that blue LEDs in combination with red LEDs and high-intensity green LEDs are more efficient for plants microgreen growth. Providing green lighting within the growing conditions increases the biomass growth, while, increasing the blue light ratio had a passive response to the nutritional profile in *Brassica* microgreens.

Many reports demonstrated the positive influence of the red:blue lighting on plant growth and photosynthesis (Potter et al. 1999, Tarakanov et al. 2012, Yano and Fujiwara 2012, Kang et al. 2013, Samuolienė et al. 2013a, Dong et al. 2014, Brazaitytė et al. 2015). Furthermore, a red, blue and green light combination is an effective source for photosynthesis (Muneer et al. 2014).

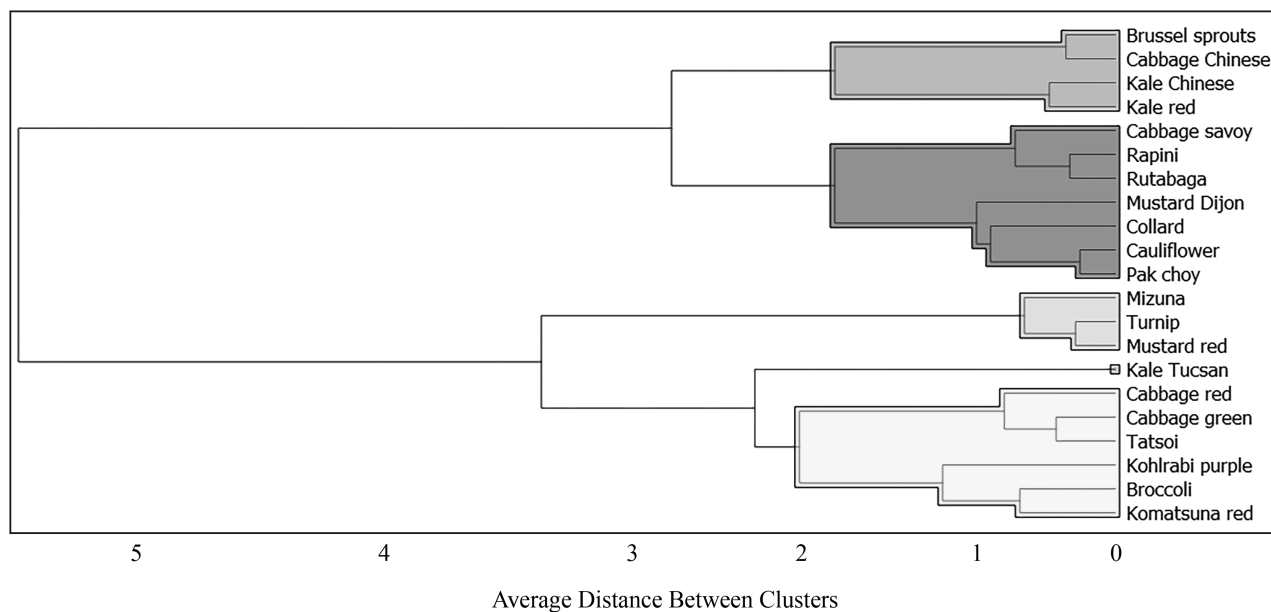


Fig. 7. The average-linkage on the normalized data sets of mineral composition and vitamin and carotenoid concentrations corresponding to the actual FW by means of the hierarchical method using growth and morphology measurements data of 21 varieties *Brassica* microgreens grown under LEDs ratio (%) of red:green:blue 70:10:20 ($R_{70}:G_{10}:B_{20}$). The complete profile of the highest cluster value (yellow cluster) microgreens presented in Table 2.

Table 2. Growth, and nutritional composition of highest *Brassica* microgreens grown under LEDs ratio (%) of red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀). A list of the seven brassica microgreens is exported from the hierarchical cluster analysis (Fig. 7).

	Kohlrabi purple	Cabbage red	Broccoli	Kale Tucsan	Komatsuna red	Tatsoi	Cabbage green
Hypocotyl length (mm)	46	47	42	49	44	46	45
Leaf area (cm ²)	1.82	1.93	1.65	2.02	1.75	1.86	1.83
Fresh weight (g)	0.44	0.46	0.42	0.47	0.41	0.45	0.44
Dry weight (%)	6.65	6.18	6.55	6.90	6.57	6.34	6.25
P (mg/100 g FW)	68	62	59	63	66	64	62
K (mg/100 g FW)	322	224	319	280	320	300	183
Ca (mg/100 g FW)	65	84	92	55	53	44	87
Na (mg/100 g FW)	46	40	50	46	28	35	69
Fe (mg/100 g FW)	0.77	0.69	0.74	0.76	0.76	0.65	0.67
Zn (mg/100 g FW)	0.43	0.40	0.42	0.38	0.38	0.41	0.33
Cu (mg/100 g FW)	0.08	0.10	0.11	0.07	0.05	0.08	0.06
Mn (mg/100 g FW)	0.39	0.35	0.41	0.46	0.34	0.35	0.37
Phylloquinone (μg/g FW)	2.6	2.3	2.0	1.7	2.2	1.5	1.4
α-Tocopherol (mg/100 g FW)	17.6	29.5	17.3	19.4	25.0	29.3	14.3
Total ascorbic acid (mg/100 g FW)	77.1	127.4	84.6	73.2	97.5	99.5	118.9
β-Carotene (mg/100 g FW)	6.6	9.9	6.9	5.4	7.1	10.6	9.6

Consequently, the supply of green light to the red and blue LEDs has a significant impact on lettuce leaves growth and photosynthetic rate compared with the red and blue LEDs only (Kim et al. 2004, Kim et al. 2006). It appears that blue and red light enhances the anthocyanins accumulation in leaves, while green light stimulates phytochrome, shifting the active pool of Type I and Type II phytochromes to include reverse accumulation of anthocyanins (Carvalho and Folta 2016).

Consequently, we demonstrated the positive influence of providing green light for improving the growth and morphology of microgreens. It is reported that HL and LA of kohlrabi, mizuna and mustard were increased when grown under green light R₇₄:G₁₈:B₈ compared with the R₈₇:B₁₃, while FW and DW% were greater in microgreens when providing green light than blue/red combination (Gerovac et al. 2016). Moreover, FW of broccoli microgreens grown under light ratios of R₈₅:G₁₀:B₅ and R₈₀:B₂₀ were higher than under R₇₀:G₁₀:B₂₀ (Kopsell et al. 2014). The same influence was observed on chlorophyll content, which improved significantly in plants grown under additional green light (Samuolienė et al. 2013b, Gerovac et al. 2016).

The reduction in the growth parameters due to the increase of blue lighting was reported. It was found that the hypocotyl elongation of kohlrabi, mizuna, mustard was decreased under the red:blue light combination due to the inhibition of the gibberellins (GA), which inhibit the hypocotyl elongation (Potter et al. 1999).

Growing microgreens plants under blue LEDs R₂₀:B₈₀ in our study enhanced the minerals composition and the vitamin content accompanied by a decrease of the growth yield compared with the green LEDs R₇₀:G₁₀:B₂₀. It is reported that broccoli microgreens grown under

blue light (R₀:B₁₀₀) produced higher and more nutrient-dense microgreens (Kopsell and Sams 2013, Kopsell et al. 2014).

Comparing the LEDs lighting ratios to find proper growing conditions, we linked the nutritional profile with the actual growth yield. We found that green LEDs R₇₀:G₁₀:B₂₀ had a positive influence on the yield, and resulted in higher mineral and vitamin concentrations due to the high growth yield. The positive increase in the mineral and vitamin contents under blue LED treatment was accompanied by reduced yield, and therefore not as advantageous.

In conclusion, the assessment of the growth and nutritional profile of 21 *Brassica* microgreens grown under LEDs technology provided a satisfactory examination of the growing condition of microgreens. Providing green lighting ratio of R₇₀:G₁₀:B₂₀ showed a positive influence on the growing microgreens growth and morphology. Interestingly, Kohlrabi purple, Cabbage red, Broccoli, Kale Tucsan, Komatsuna red, Tatsoi and Cabbage green are presented as the top microgreen's candidates of our study assessment, that serve as a life support component in limited space-based conditions and controlled environment growth chambers.

Author contributions

K.Y.K., A.A.N conceived and designed the experiments. K.Y.K., A.A.E, A.A.N, M.A.S.A. and S.J. L.Z. performed the experiment. K.Y.K., D.A.M., A.A.S, S.M.A and S.Y. M analyzed the data. K.Y.K., A.A.N. wrote the manuscript. R.H., M.A.E. and M. F. R. contributed to the manuscript writing and revision. All authors revised the manuscript.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Limit of detection of LOD (LOD/mg/kg DW) and limit of quantification (LOQ/mg/kg DW) based on background equivalent concentration.

Table S2. Growth and morphological measurements [hypocotyl length (mm) and leaf area (cm²)] of 21 varieties of *Brassica* microgreens representing five species grown under different light-emitting diodes (LEDs) ratio (%) of red: blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red: green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table S3. Growth parameters [fresh weight (g) and dry weight (%)] of 21 varieties of *Brassica* microgreens representing 5 species grown under different light-emitting diodes (LEDs) ratio (%) of red:blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀).

Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table S4. Mineral composition and content of macroelements; P and K (mg/100 g FW) of 21 varieties of *Brassica* microgreens representing 5 species grown under different light-emitting diodes (LEDs) ratio (%) of red: blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table S5. Mineral composition and content of macroelements; Ca and Mg (mg/100 g FW) of 21 varieties of *Brassica* microgreens representing 5 species grown under different light-emitting diodes (LEDs) ratio (%) of red: blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table S6. Mineral composition and content of microelements; Fe and Zn (mg/100 g FW) of 21 varieties of *Brassica* microgreens representing 5 species grown under different light-emitting diodes (LEDs) ratio (%) of red: blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Mean \pm SE values are based on a representative sample from each treatment across three experimental

replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table S7. Mineral composition and content of microelements; Cu and Mn (mg/100 g FW) of 21 varieties of *Brassica* microgreens representing 5 species grown under different light-emitting diodes (LEDs) ratio (%) of red: blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table S8. Vitamins concentrations [phylloquinone (μ g/g FW) and α -tocopherol (mg/100 g FW)] of 21 varieties of *Brassica* microgreens representing 5 species grown under different light-emitting diodes (LEDs) ratio (%) of red: blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table S9. Vitamin and carotenoid concentrations [Total ascorbic acid (mg/100 g FW) and β -carotene (mg/100 g FW)] of 21 varieties of *Brassica* microgreens representing 5 species grown under different light-emitting diodes (LEDs) ratio (%) of red: blue 80:20 (R₈₀:B₂₀), red: blue 20:80 (R₂₀:B₈₀), red:green:blue 70:10:20 (R₇₀:G₁₀:B₂₀) or red:green:blue 20:10:20 (R₂₀:G₁₀:B₇₀). Mean \pm SE values are based on a representative sample from each treatment across three experimental replications. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.