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Authors

Qian, Yufei
Hibbert, Lauren E
Milner, Suzanne
et al.

Publication Date

2022-06-01

DOI

10.1016/j.scienta.2022.111068

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Scientia Horticulturae 300 (2022) 111068

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Improved yield and health benefits of watercress grown in an indoor vertical farm

Yufei Qian [a], Lauren E. Hibbert [a,b], Suzanne Milner [a], Ella Katz [a], Daniel J. Kliebenstein [a], Gail Taylor [a,b,*]

[a] Department of Plant Sciences, UC Davis, Davis, CA, 95616, USA
[b] School of Biological Sciences, University of Southampton, Southampton, Hampshire, SO17 1BJ, UK

ARTICLE INFO

Keywords:
Vertical farm
Glucosinolates
Yield
Nutrition
Controlled environment agriculture
Leafy green

ABSTRACT

Watercress (Nasturtium officinale R. Br.) is a highly nutritious leafy crop with a rich health-related phytonutrient profile including secondary plant metabolites such as glucosinolates. This semi-aquatic crop is well-suited for indoor hydroponic cultivation and has a growing market for its distinctive peppery taste and health benefits. We describe crop trials in three contrasting environments and report the changes to plant morphology, yield, and nutritional profile under varying blue light treatments. Yield and glucosinolate quantity were significantly increased in a vertical farm system enriched in blue light, relative to field-grown crops. Additionally, PBITC, a glucosinolate not previously identified in field-grown watercress, was found in plants grown in a vertical farm. This work demonstrates the great potential of watercress in a new era of controlled environment agriculture to deliver improved health benefits to customers.

1. Introduction

Watercress is a leafy-green crop in the Brassicaceae family, consumed widely across the world for its peppery taste and known to be the most nutrient dense salad leaf (Di Noia, 2014). The peppery taste is the result of high concentrations of glucosinolates (GLSs) - phytochemicals which can be hydrolyzed to isothiocyanates (ITCs) upon plant tissue damage, such as chewing, known for their potent anticancer (Giallourou et al., 2019), anti-inflammatory (Cheung et al., 2009), and antioxidant effects (Azarmehr et al., 2019; Bahramikia and Yazdanparast, 2010) that are beneficial to human health. Although ITCs are the main products of digestion depending on pH, metal ions, and other epithiospecifier proteins, nitriles can also be formed through GLS break-down and they too may have chemopreventitive properties (Bones and Rossiter, 2006; Ishida et al., 2014).

Watercress is high value horticultural crop. A specialty leafy vegetable, with a growing area of 282 ha in the US, with 75 ha of production in California, compared to 58 ha in the UK (DEFRA, 2019; USDA, 2017). It is also a high-value horticultural crop in the UK, with the market value of £8.90 per kg compared to £4.97 per kg for mixed baby leaf salad bags and represents a total value of £15 million per year (DEFRA, 2019, 2020). Watercress is traditionally grown in outdoor aquatic systems, but there is increasing interest in its suitability for indoor hydroponic systems, such as in vertical farms (VF; SharathKumar et al., 2020). VF utilize hydroponic or aeroponic systems that allow plant stacking in multiple vertical or horizontal layers increasing the effective use of space (Avgoustaki and Xydis, 2020; Despommier, 2013) and other resources, particularly water (Kozai, 2013). Indoor vertical agriculture is well-suited to the production of leafy greens. Their fast growth rate, high harvest index, low photosynthetic energy demand and compact shape make them ideal for indoor farming technologies (Shamshiri et al., 2018). VFs have multi-layered indoor crop production space with the use of artificial lights (usually LEDs) and soilless cultivation systems. With the capacity to control lighting, ventilation, irrigation, nutrient levels, and abiotic stress, VFs offer the potential of high and predictable yields and uniform produce alongside reduced water use and often no pesticide applications whatsoever (van Delden et al., 2021). The future of indoor food production is likely to include other high-value horticultural crops such as several leafy greens, culinary herbs, strawberries, and flowers. Breeding targets for these crops include short life cycles, low energy demands, improved yield (high partitioning of assimilates to marketable organs), small root systems, as well novel sensory and nutritional profiles (Folta, 2019).

VF systems are gaining traction for commercial scale cultivation,

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[*] Corresponding author.
E-mail address: gtaylor@ucdavis.edu (G. Taylor).

https://doi.org/10.1016/j.scienta.2022.111068
Received 7 December 2021; Received in revised form 14 February 2022; Accepted 15 March 2022
Available online 26 March 2022
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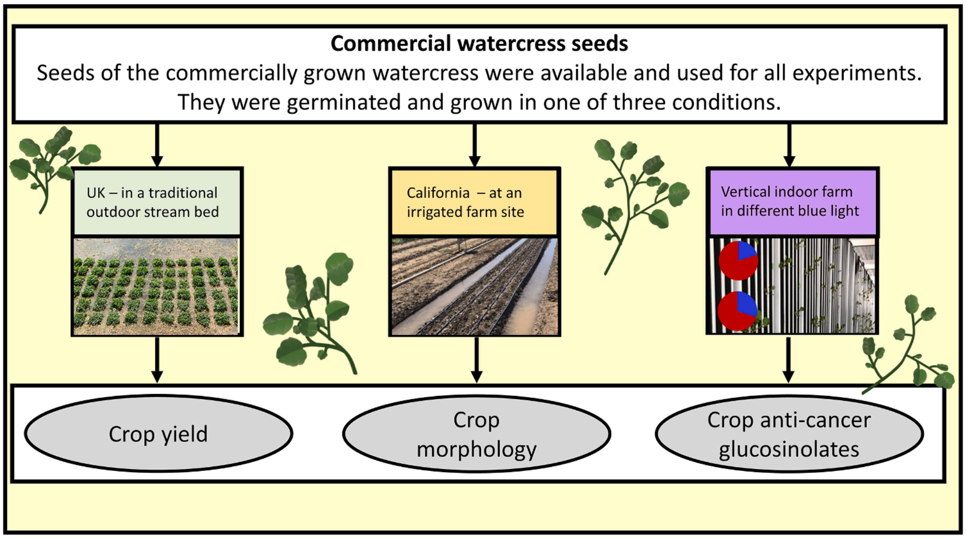

Fig. 1. A flowchart of the research scheme, from plant material to data collection, for each of the three contrasting growing systems.

partly due to their ability to deliver locally-grown food to urban areas, with lower environmental costs and also to deliver food in locations where fresh produce cannot be easily grown (Al-Kodmany, 2018). These systems also offer a unique opportunity to tailor crop characteristics to changing consumer preferences by altering environmental conditions such as light quality for example (Dou et al., 2020; Larsen et al., 2020), where blue light has been used to increase the glucosinolate content of several Brassica species including, pak choi and watercress (Lam et al., 2021; Zheng et al., 2018). Here we investigate differences in yield, morphology, and glucosinolate content of watercress grown under three different cultivation systems (a traditional outdoor aquatic, a soil-based irrigated field, and an indoor VF with varying blue light levels). This research provides foundation information to suggest that high yield watercress crop production is possible in vertical farming systems and that watercress quality may be further enhanced for improved anti-cancer characteristics.

2. Materials and methods

2.1. Plant material

A commercial watercress (Nasturtium *officinale* R. Br.; Brassicaceae) line with low heterozygosity and high vigor was cultivated from seeds germinated in plug trays until roots were well-established (21 days), and then transplanted into one of three experimental sites. Seeds were germinated in a controlled-environment greenhouse (23 °C day/21 °C night, 12 h day length) and planted on Pro-Mix HP (CA site), 80:20 Baltic white: Baltic black peat (UK site), or rockwool (indoor vertical farm site) in plug trays.

2.2. Growth environments

Three contrasting cultivation systems (Fig. 1) were used for trials (i) A trial in a UK traditional watercress farm in spring-fed water, (ii) A trial in a California (CA) soil-grown, irrigated farm and (iii) A trial in a vertical indoor farm with two levels of red:blue light ratios (20% and 30% blue).

2.2.1. UK site

From May to July 2019, the UK field trial was conducted on a traditional outdoor aquatic-based commercial farm (51°11′42.9″N 1°32′12.9″W; Hampshire, UK). Watercress seedlings were treated according to commercial watercress fertilizer regimes. Irrigation was supplied continuously in the form of running borehole water through the bed at a constant temperature of approximately 12 °C.

2.2.2. CA site

From May to July 2019, field trial was conducted on a soil-based experimental field at the University of California Davis Vegetable Crops headquarters (38°33′8.875′’N, 121°43′13.369′’W; Davis, USA). Four drip tapes were installed evenly in this bed and the water applied at 05:00, 10:00, 15:00, and 20:00 for 30 min to create a wet field. No fertilizer was applied to the field during this experiment.

2.2.3. Indoor vertical farm site

From November 2020 to January 2021, this commercial line was grown in the UCD indoor VF (model: Greenery S, manufacturer: Freight Farm, MA, USA). This indoor farm has four walls of growth panels, hanging side by side with emitters at the top to supply water and nutrients. Light treatment 1 (20% blue light) and light treatment 2 (30% blue light) were applied after transplanting and stopped one day before harvesting. Growth light intensity was at 220 $\mu mol/m^2/s$ photosynthetic photon flux density (PPFD) with both red and blue LEDs, 38 $\mu mol/m^2/s$ under blue only and 187 $\mu mol/m^2/s$ under red light only. Fertilizer containing macro and micronutrients was dosed to maintain an electrical conductivity at 400–800 μ.

2.3. Phenotyping

Fresh aboveground weight and main stem diameter (at midpoint) of each individual plant was recorded. Images of dissected plants were analyzed using ImageJ version 1.52d (National Institutes of Health, USA) for leaf area. The shoot was bagged and oven-dried at 80 °C for 48 h for dry weight analysis.

Glucosinolates were measured as previously described (Kliebenstein et al., 2001). Individual desulfo-GLSs within each sample were separated and detected by HPLC-DAD, identified, quantified by comparison to standard curves from purified compounds, and further normalized to equalize the fresh weight. Pure aromatic glucosinolate known to be in watercress was obtained from Extrasynthese (Genay, France) to add to the existing collection of standards.

2.4. Statistical analysis

All statistical analyses were performed in R (version 4.1.1) and R studio (version 1.4.1717). One-way ANOVAs were performed and if

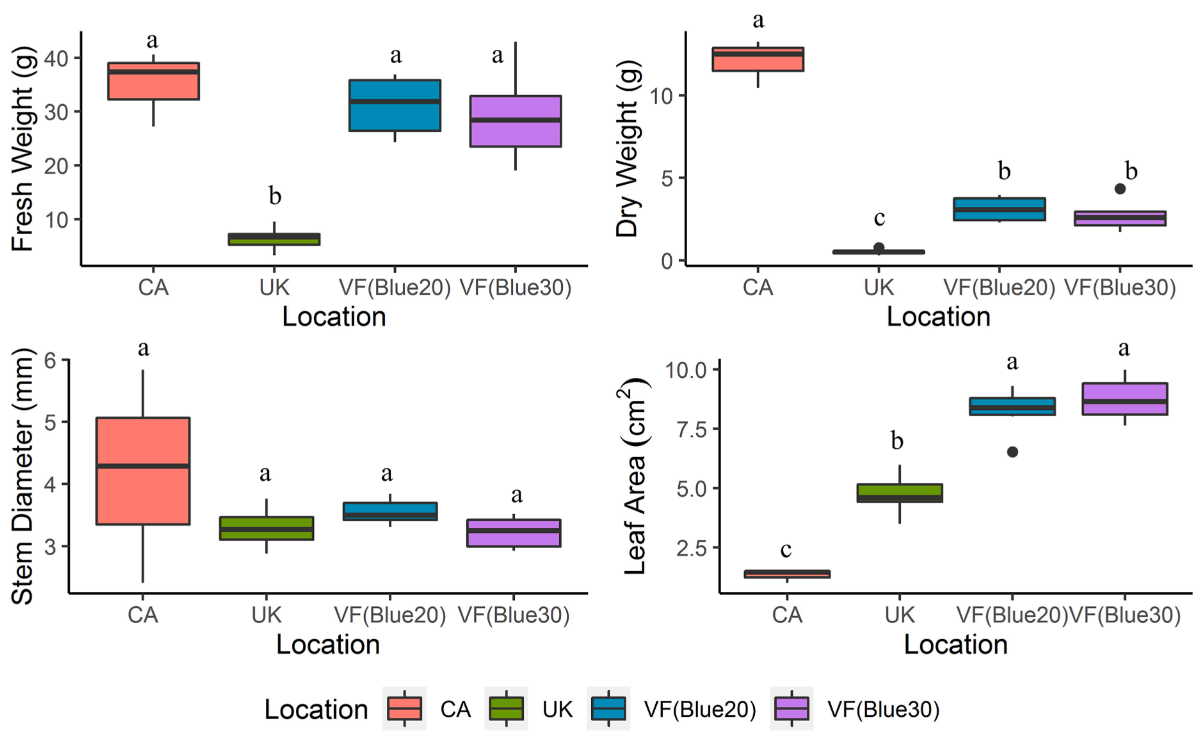

Fig. 2. Phenotypic assessments in three locations for fresh weight, dry weight, leaf area, and main stem diameter (clockwise). Boxplots represent median and interquartile range for the investigated trait at each location, with red, green, blue, and purple color for California, UK, and indoor vertical farm environments with 20% and 30% blue light respectively. Different letter groups report significant differences according to Tukey HSD test results at $p \leq 0.05$.

there was significance found in the main effects, then Tukey HSD post-hoc tests were used to test for significant differences between group means (α=0.05). All values were reported as means and standard errors, unless indicated otherwise.

3. Results

We hypothesized that optimized light and temperature regimes in the VF system was likely to result in higher yield and glucosinolate content, as well as less variability between plants due to tightly controlled conditions. Comparing across growth environments, four morphological traits were grouped (fresh weight, dry weight, stem diameter, and leaf area) by locations. Plants showed high plasticity between environments.

Watercress plants exhibited considerably different growth morphologies depending on the growing system (Fig. 2). Despite being the system currently used for commercial cultivation, watercress yield was lowest in the UK trial; fresh weight (FW) was significantly higher in the CA and VF trials ($p < 0.001$). Plants grown in CA, however had less palatable "woody" growth types, with smaller leaves, illustrated by a significantly higher dry weight compared to UK and VF (DW; $p < 0.001$). Stem diameter was not affected by growth environments ($p > 0.05$). Most importantly, the harvestable part of the plant, leaf area, differed significantly between environments ($p < 0.001$), with the smallest leaves produced in CA and the largest in the VF trials: mean individual leaf area (cm^2) for CA, UK VFBlue20 and VFBlue30 was 1.33 ± 0.46, 4.69 ± 0.23, 8.25 ± 0.33, and 8.75 ± 0.33, respectively. Increasing blue light in the VF treatments had no impact on morphology. Also, not all morphological traits changed unidirectionally (Fig. 3).

Glucosinolate composition and quantity was significantly impacted by growth environments. Short chain aliphatic GLSs (6MSO and 7MSO) and longer chain aliphatic GLSs (8MSO) were all found at higher concentrations when grown in the VF compared to the UK or CA. Low concentrations of Indole GLSs (I3M) were found in all watercress (0.009 ± 0.001 nmol/mg), CA (0.006 ± 0.001 nmol/mg), VFBlue20 (0.004 ± 0.0007 nmol/mg), and VFBlue30 (0.002 ± 0.0007 nmol/mg). Conversely, the most abundant aromatic GLS compound in watercress, gluconasturiin (2-phenyl-ethyl glucosinolate (PEGLS)), had mean concentrations of 0.56 ± 0.17 nmol/mg (CA), 1.71 ± 0.17 nmol/mg (UK), 1.98 ± 0.12 nmol/mg (VFBlue20), and 2.18 ± 0.12 (VFBlue30). The UK-grown watercress had the lowest PEGLS concentration compared to the other sites. GLS quality was altered in the red:blue light treatment of the VF, and a new compound PBGLS (4-phenyl-butyl GLS), an alkyl-glucosinolate homologous to PEGLS with a longer alkyl chain, was detected in the VF that was not apparent for outdoor grown watercress in either CA or the UK (VFBlue20 0.065 ± 0.003 nmol/mg; VFBlue30 0.074±0.003 nmol/mg).

Interestingly, between the two light regimes of the VF trials, neither GLS quality nor quantity differed, but total GLS was altered between UK, CA, and VFBlue30 treatment, indicating that the GLS quantity profile changes under distinctive environments (Table. 1).

4. Discussion

We have shown that the quality and yield of the leafy green salad crop watercress can be significantly improved by growth in an indoor vertical hydroponic system, enriched in blue light.

The CDC ranked watercress as the most nutrient dense crop based on the content of 17 nutrients (common to the tested produce) that are associated with reducing chronic disease risk (Di Noia, 2014). Our results show the yield and nutrient content of watercress can be enhanced even further by utilizing a novel vertical indoor growing environment other than the current commercial system used in the UK. Yield increases may be explained by the ability to tightly control environmental conditions in the VF that generate a consistent optimal nutrient and temperature environment. The increase in glucosinolate content from UK to CA is probably explained by heat stress in CA, with the maximum temperature recorded at the CA site at 43.8 °C compared to 30.9 °C for the UK. Glucosinolate accumulation is associated with improved heat and drought stress tolerance in Arabidopsis and increases in GLSs are

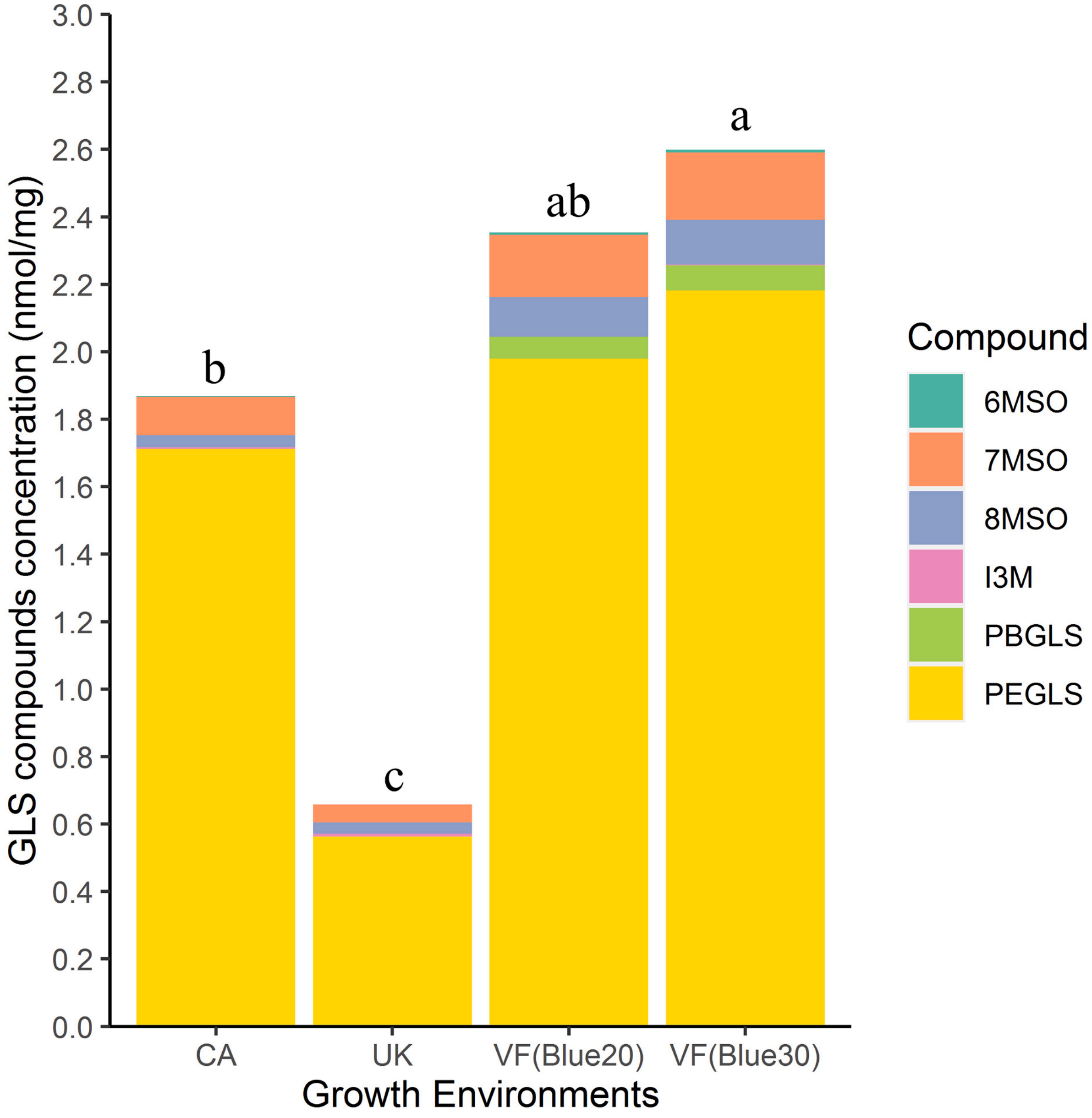

Fig. 3. Stacked bar chart with absolute value of each GLS compound from three locations. The primary component is aromatic GLS (PEGLS, 2-Phenyl-ethyl GLS) following by aliphatic GLSs (6MSO,7MSO, and 8MSO). 4-Phenyl-butyl GLS (PBGLS) was only found in the VF growth environment. Letter group on top of each stacked bar chart represents the total range test result.

Table 1
Tukey HSD result on each and total GLS compounds across locations. Different letter groups indicate statistically significant differences at $p \leq 0.05$.

	UK	CA	VF (Blue 20)	VF (Blue 30)
6MSO	b	b	a	a
7MSO	b	b	a	a
8MSO	b	b	a	a
I3M	a	ab	c	bc
PEGLS	b	a	a	a
PBGLS	b	b	a	a
Total	c	b	ab	a

observed in heat-stressed *Brassica rapa* (Hara et al., 2013; Salehin et al., 2019; Shawon et al., 2020). Increases observed in GLS content in VF can be explained by prolonged blue light exposure and a longer growth period (Martínez-Ballesta et al., 2013). The mechanism of different LEDs on GLS biosynthesis regulations still remain unclear, but a short-duration blue light photoperiod increased the total aliphatic GLSs in broccoli (Kopsell and Sams, 2013; Loi et al., 2021). A similar result from a genome wide association mapping of Arabidopsis also revealed that blue light controlled GLS accumulation by altering the PHOT1/-PHOT2 blue light receptors (Chan et al., 2011).

Increasing blue light in the VF increased total GLSs content and although not statistically significant, it confirms the study by Chen et al. (2021) that showed increased GLSs content with increased blue light. Rosa et al. showed that GLS concentrations are more sensitive to the effect of temperature than of photoperiod (Rosa et al., 1994) and this is consistent with our results in total GLSs between the UK and CA sites.

Our results support the idea that indoor farm cultivation is effective in promoting health-beneficial chemical properties. Watercress produced PBGLS in both the VF treatments, but this compound was not detected in either the UK or CA trials. PBGLS strengthens the nutrient profile of watercress. PEITC derived from PEGLS has already been proven to be an extremely effective naturally-occurring dietary isothiocyanates against cancer (Gupta et al., 2014). Inhibitory potency increases several-fold when the glucosinolate alkyl chain gets longer (Chung et al., 1992), suggesting that PBITC, with its elongated alkyl chain compared to PEITC, may contribute an additional health benefit to this super food, although this remains to be proven.

It is evident that watercress is particularly well-suited for indoor hydroponic growing systems, where plants exhibited the highest yielding leafy growth with improved nutritional profiles, ideal for consumer preferences. Altering the blue:red light ratio may further enhance the anti-cancer properties of this highly nutritious salad crop, but further studies are required to hone the light recipe for indoor cultivation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

YQ conducted the US and VF trials (design, field work, and raw data collection). LH completed the UK field trial (design, field work, and raw data collection). SM set up the VF facility and conceptualized the experimental design of the VF trial. Formal analysis of data and figure productions from USA, UK, and VF trials were achieved by YQ. EK generated the HPLC data and DJK provided the equipment for the HPLC analysis. Both LH and YQ contributed to the paper drafting and editing equally. GT conceptualized and led all aspects of the research. All authors commented on the draft manuscript.

This work was funded by University of California, Davis as part of the John B. Orr Endowment to the research program of Gail Taylor.

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