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Recent advances in research and development for vegetable crops under protected cultivation

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Giao Nguyen and Zora Singh

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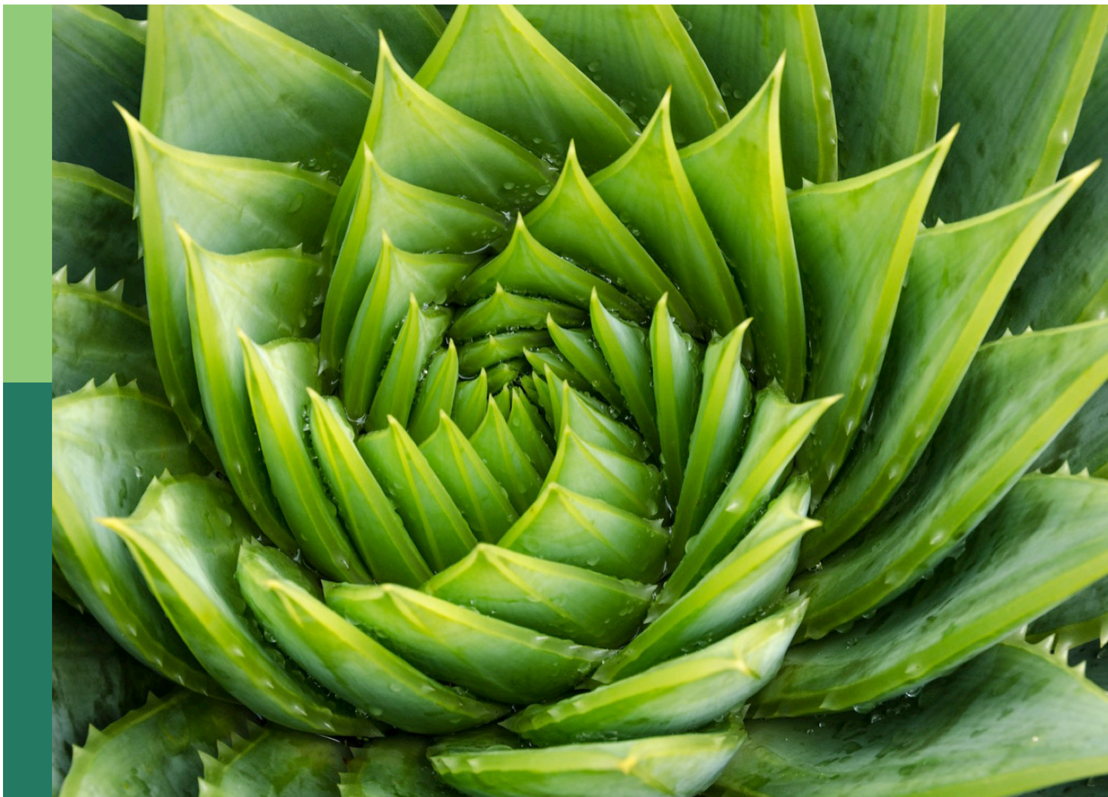

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Recent advances in research and development for vegetable crops under protected cultivation

Topic editors
Giao Nguyen — Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia
Zora Singh — Edith Cowan University, Australia

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EDITED AND REVIEWED BY
Leo Marcelis,
Wageningen University and Research,
Netherlands

*CORRESPONDENCE
Giao N. Nguyen
✉ giao.nguyen@dpird.wa.gov.au

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Editorial: Recent advances in research and development for vegetable crops under protected cultivation

Giao N. Nguyen[1]* and Zora Singh[2]

[1]Department of Primary Industries and Regional Development, Carnarvon, WA, Australia,
[2]Horticulture, School of Science, Edith Cowan University, Joondalup, WA, Australia

KEYWORDS

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Editorial on the Research Topic
Recent advances in research and development for vegetable crops under protected cultivation

Protected cultivation is the production of horticultural crops including vegetables under structures such as forced-ventilated greenhouses, shade net houses, poly houses, net houses, high and low plastic tunnels, where the external growing environment can be controlled or modified to suit crop growth requirements. In regions where the environmental conditions such as weather, soil and water are not suitable to grow specific vegetables, or in urban areas with limited arable land, this farming system is very important to ensure high, stable productivity and good quality vegetables. This enables a stable supply for the market all year round, bringing high economic return to growers. This research area has been receiving much attention from the commercial vegetable production sector and scientific community. With the aim to contribute to the understanding of this research area, this Research Topic was designed to collect the latest scientific research and development in protected cropping for commercial vegetable production. Eight original research and review articles were already published with the invaluable contribution of 54 authors coming from 20 research institutions in 7 countries. This editorial summarises key highlights from the articles contributed to this theme.

To gain an insight into current vegetable production systems, Ahmed et al. provided a comprehensive review on various aspects of modern vegetable production. The authors analysed and discussed the transition from traditional cultivation to modern greenhouse vegetable production methods. The review emphasised the role of scientific research and advanced production support tools for precise fertigation, irrigation and integrated pest management. The utilisation of drones, robots and digital monitoring systems for vegetable production was also intensively discussed.

Having suitable, affordable and environmentally friendly growing media substrates for specific vegetable crops for high yield and good quality is critical for a successful soilless growing method under protected cultivation. Although peatmoss is widely used for as an ideal substrate for hydroponic vegetables, it raises a copious number of environmental concerns. To this purpose, Yu et al. dedicated their work to review the use of biochar as an alternative subtract to peatmoss. They suggested that biochar can be used for greenhouse and nursery production and brings environmental and economic benefits. Under their view, biochar is a source of renewable materials that can be created from many different materials available in nature. Further on this subject, Adamczewska-Sowin´ska et al., reported on a more suitable material, willow tree chips which are environmentally friendly and abundant in nature, as an alternative substrate for growing cucumbers. The authors evaluated a total of 29 mixtures using potting materials such as willow, peat, biochar, and basalt meal and found that willow chips could be used as a partial substitute for environmentally harmful peat.

Modifying unfavourable environmental conditions such as light, temperature and humidity to facilitate crop growth is critical for vegetable production under protected cropping. In this Research Topic, four papers provided an insight the role of light and its interaction with environmental factors to cause an impact on crop metabolism, yield and quality under this theme. Using appropriate materials to build a greenhouse is extremely important, ensuring light and temperature for plant photosynthesis, while saving energy to reduce costs and increase the competitiveness of fresh produce. Maier et al. conducted a thorough investigation on the effects of light blocking film to the growth, development and yield of capsicum under various seasonal conditions. Their research findings proved that the choice of right roof cover when constructing a greenhouse is essential to ensure that adequate light quantity and quality can be achieved for optimum crop growth. Likewise, Ramezani et al. highlighted the role of different light spectra generated by different light-emitting-diodes and nitrogen supplementation on spinach in a controlled environment agriculture platforms. The authors provided an insight into the interaction between light spectra combinations with an emphasis on green light and nitrogen modulating the spinach yield and quality. These results again confirmed the importance of light source selection for protected cropping. The importance of the supplementary light source was again reported by an interesting study of Moratiel et al., where the interplay between light spectra, their intensities and carbon dioxide concentrations on the net photosynthesis of tomatoes seedlings was thoroughly investigated. The results from this study showed that the properties of the spectrum are essentially crucial under low light conditions to achieve the optimum crop's net photosynthesis. The study of Begum et al. on chilling resistant sweet basil further emphasized on the importance of the light components. The authors demonstrated that a short duration of far-red light supplement four days before harvesting would alter several biochemical pathways and enhance the cold tolerance of sweet basil. These findings could be useful for a large-scale commercial basil production to reduce chilling injuries and improving self-life of fresh basil.

Maintaining optimal root zone temperatures are critical for the nutrient uptake of vegetable cultivation using soilless method since fluctuations in nutrient solution temperatures will cause changes in other attributes such as electrical conductivity and pH, which affect nutrient absorption at the root zone. Developing a cost-effective and energy efficient method that can maintain nutrient solution temperatures is essential. Nisar et al. designed and compared of the efficacy of four energy saving (non-electric) cooling methods for several vegetables in open-air hydroponics. The results showed that the cooling setup III outperformed the other three methods, and it could reduce the nutrient solution temperature up to 193%. Intuitively this method can potentially be applied for hydroponic systems in protected cropping to stabilize root zone temperatures while saving energy consumption which is usually a major cost for commercial vegetable production.

We hope that eight articles published in this Research Topic will provide the readers with up-to-date content related to vegetable production under protected cultivation. Nevertheless, further studies on greenhouse construction materials, and optimal designs suitable for various ecozones, that save energy and reduce production costs would be worth investigating. The selection of suitable crop varieties for protected cultivation for optimal yield and premium quality together with an integrated pest management system, using minimal pesticides, extending postharvest life and minimising postharvest losses also needs more attention from the vegetable scientific community. Furthermore, advanced agronomy methods such as using closed hydroponics system where the run to wastewater can be efficiently recycled to optimise water and fertilizer use could be interesting Research Topics in near future.

Author contributions

GN: Writing – original draft, Writing – review & editing. ZS: Writing – review & editing.

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Conflict of interest

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

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EDITED BY
V. Mohan Murali Achary,
International Centre for Genetic Engineering and Biotechnology, India

REVIEWED BY
Giancarlo Fascella,
Council for Agricultural and Economics Research (CREA), Italy
Junaid Ghani,
University of Bologna, Italy

*CORRESPONDENCE
Ping Yu
✉ pingyu@uga.edu

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Alleviate environmental concerns with biochar as a container substrate: a review

Ping Yu[1]*, Kuan Qin[1], Genhua Niu[2] and Mengmeng Gu[3]

[1]Department of Horticulture, University of Georgia, Griffin, GA, United States, [2]AgriLife Research Center, Department of Horticultural Sciences, Texas A&M University, Dallas, TX, United States, [3]Department of Horticulture and Architecture, Colorado State University, Fort Collins, CO, United States

Peat moss has desirable properties as a container substrate, however, harvesting it from peatland for greenhouse/nursery production use has disturbed peatland ecosystem and caused numerous environmental concerns. More recently, many nations have taken actions to reduce or ban peat moss production to reach the carbon neutral goal and address the environmental concerns. Also, the overuse of fertilizers and pesticides with peat moss in greenhouse/nursery production adds extra environmental and economic issues. Thus, it is urgent to find a peat moss replacement as a container substrate for greenhouse/nursery production. Biochar, a carbon-rich material with porous structure produced by the thermo-chemical decomposition of biomass in an oxygen-limited or oxygen-depleted atmosphere, has drawn researchers' attention for the past two decades. Using biochar to replace peat moss as a container substrate for greenhouse/nursery production could provide environmental and economic benefits. Biochar could be derived from various feedstocks that are regenerated faster than peat moss, and biochar possesses price advantages over peat moss when local feedstock is available. Certain types of biochar can provide nutrients, accelerate nutrient adsorption, and suppress certain pathogens, which end up with reduced fertilizer and pesticide usage and leaching. However, among the 36,474 publications on biochar, 1,457 focused on using biochar as a container substrate, and only 68 were used to replace peat moss as a container substrate component. This study provides a review for the environmental and economic concerns associated with peat moss and discussed using biochar as a peat moss alternative to alleviate these concerns.

KEYWORDS

peat moss, substrate properties, pathogens, economic benefits, potted plant

1 Introduction

Peatlands contribute vital ecological services such as storing organic carbon (C) and nitrogen (N), regulating water, influencing methane (CH_4), and providing habitats (Leifeld and Menichetti, 2018; Humpenöder et al., 2020). Peatlands occupied around 4% of the terrestrial surface but stored 644 Gt of C or 21% of the global total soil organic C stock (Yu et al., 2010; Scharlemann et al., 2014; Dargie et al., 2017; Leifeld and Menichetti, 2018). Northern peatlands alone store 17 Gt N, and for well-grown sphagnum peatlands, one single sphagnum farming site takes up N at 35~56 kg ha^{-1} yr^{-1} (Temmink et al., 2017; Hugelius et al., 2020). By regulating water flows, peatlands help minimize the risk of flooding and drought and prevent seawater intrusion (Rizzuti et al., 2004). In the peatland system, up to 90% of biologically CH_4 produced is consumed due to activities of methanogens and methanotrophs (Liebner et al., 2011). Peatlands also provide precious habitats for different wild animals (Alexander et al., 2008).

Harvesting peat moss, a commonly used container substrate in horticulture, has caused numerous environmental concerns. Large scale peatlands drainage caused carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions more than 2 Gt CO_2-eq yr^{-1}. The CO_2 emissions from the drained peatlands are estimated at 1.3 Gt CO_2 annually, which is equivalent to 5.6% of the global anthropogenic CO_2 emissions (Nature, 2017). In addition, the drainage of peatland, with other gas and fuels extractions, contributed 23% of the total CH_4 budget of 500 to 600 tera gram per annum (Reumer et al., 2018), and increased the total CH_4 emissions from 334 Tg yr^{-1} to 366 Tg yr^{-1} (Saunois et al., 2020). Peatland extraction reduced surface and groundwater quality, and increased land compaction (Temmink et al., 2017). Moreover, peat extraction has caused 15% of global peatland habitats lost for wild animals, including Bornean Orangutans (Barthelmes, 2016; Nature, 2017; Vaughn et al., 2018). If the peatland extraction trend continues, the cumulative of greenhouse gases (GHGs) CO_2 equivalent emission would reach to 249 Gt by 2100 (Heck et al., 2021). Among the 17 United Nations Sustainable Development Goals, 8 goals are closely related to ecosystem interference and global warming (The United Nations Sustainable Development Goals, 2015). Therefore, reducing the use of peat moss and finding a peat moss replacement is necessary and urgent.

There are several potential organic materials that can be used as peat moss replacements, including coconut coir, rice hull, and wood bark. In addition to these materials, recently, biochar has received attention as a superb peat moss alternative with many advantages. Since it has been long discovered in the amazon rainforest as terra preta (black soil), biochar has been evaluated and studied from researchers in the past two decades (Denevan and Woods, 2004). Biochar is a carbon-rich material with porous structure produced by the thermo-chemical decomposition of biomass in an oxygen depleted or oxygen-limited atmosphere (Demirbas and Arin, 2002; Lehmann, 2007; Nartey and Zhao, 2014). Data from the literature were obtained from web of science database from 2010 to 2023 with searching terms such as “biochar”, “biochar container substrate”, “biochar environment”, “biochar peat moss” etc. The number of biochar-related publications increased from 76 to 3,6474 in the past two decades (Figure 1.), with its main applications being soil amendments (Lehmann et al., 2011), pollutant removal (Ahmad et al., 2014), beneficial bacterial carrier (Belonogova et al., 2018), and mitigate climate change (Woolf et al., 2010). Most of studies were either focused on increasing crop growth or reducing non-peat moss related environmental concerns such as carbon sequestration, contaminants remediation, greenhouse gas emission reduction (Das et al., 2020; Bolan et al., 2022a; Bolan et al., 2022b). There were decent number of studies concentrated on biochar production, characterization and engineering (Albert et al., 2021; Basak et al., 2022; Bolan et al., 2023). Among these 36,474 publications, 1457 focused on using biochar as a container substrate, and only 68 were used to replace peat moss as a container substrate component.

Based on the existing information, using biochar as a container substrate holds immense potential to offer substantial environmental and economic benefits for various compelling reasons. Unlike peat moss, which needs a long time to regenerate, biochar is considered as a renewable material since it can be derived from various and fast generating feedstocks (Yan et al., 2020), ranging from plant-based material such as green waste (Tian et al., 2012), wood (Vaughn et al., 2015; Gascó et al., 2018; Fascella et al., 2020; Ferlito et al., 2020), straw (Spokas et al., 2009; Spokas et al., 2010; Vaughn et al., 2013; Hansen et al., 2015; Hansen et al., 2016), bark (Hina et al., 2010), rice hull (Locke et al., 2013), wheat straw (Vaughn et al., 2013; Xu et al., 2016; Xu et al., 2016) to other sources such as deinking sludge (Méndez et al., 2015; Méndez et al., 2017). For the same reason, biochar has price advantages over peat moss, especially when biochar is made from feedstocks from local industries and farms (Yan et al., 2020). Using biochar as a peat moss replacement protects peatland from further drainage for peat moss harvesting, thus protecting peatland ecosystems and reducing GHGs emissions (Hao et al., 2010; Ro et al., 2010; Cornelissen et al., 2013; Conversa et al., 2015). Moreover, producing straw biochar and adding it into agriculture production can directly reduce CO_2 emission by 47% and 57% for rice and maize, respectively (18,479.35–37,457.66 kg) and reduce CH_4 and N_2O emission (Ji et al., 2018; Xu et al., 2018). Biochar could increase water and nutrient use efficiency, reduce fertilizer and pesticide runoff, render equivalent plant yield, thus providing both environmental and economic benefits (Guo et al., 2018a; Huang et al., 2019a; Yan et al., 2020; Yu et al., 2020a).

As such, this article discussed the use of biochar to replace peat moss as a container substrate to alleviate environmental issues by collecting exponentially increased number of publications and reviewing them to explain how the properties of biochar make it a viable alternative to peat moss, how biochar helps in reducing fertilizer pollution and the leaching of nutrients, how it addresses issues related to peatland disturbance, and how it provides potential economic benefits. This article also provides new insights into the research gap, state-of-the-art challenges of using biochar on a large scale and the possible solutions. The future research directions of using biochar as a peat moss alternative was also discussed. The structures and key points for this study are: 1) biochar has huge potential to replace peat moss as a container substrate component; 2) biochar can provide environmental and economic benefits; 3) more actions need to be taken to use biochar in horticulture area in a large scale.

2 Peat moss used as a container substrate

2.1 Properties of peat moss

Peat moss has long been widely used a container substrate due to its suitable properties, which allows it to support plants, hold nutrients, retain water, and change gases (Yeager et al., 2007; Nelson, 2012). Despite its suitable properties, peat moss could have

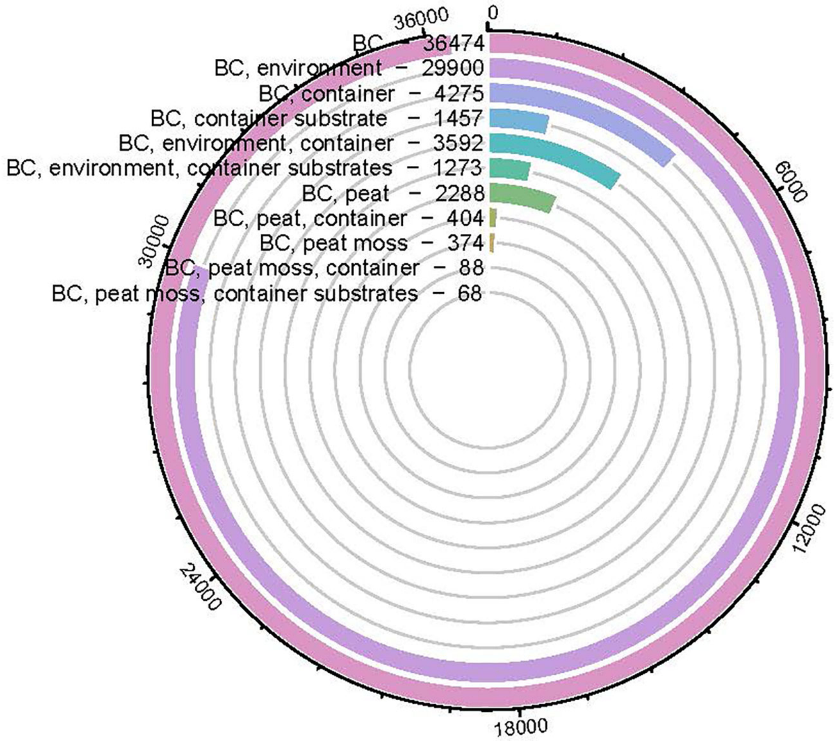

FIGURE 1
Circular bar-plot indicating the number of biochar (BC)-related articles published from 2010~2023 based on key words searching in Science Direct database.

rewetting and leaching issues (Gaudig et al., 2017; Kumar, 2017). The drying process during commercial peat moss production made it hydrophobic (Beardsell and Nichols, 1982; Gaudig et al., 2017; Kumar, 2017), and as an organic material, peat moss breaks down during greenhouse practices, which changes its hydrophobicity intensity and causes rewetting issues (Valat et al., 1991; Dekker and Ritsema, 2000). Especially after dried out, when the moisture content decreases below 20%, peat moss requires a longer time to rewet as it becomes more hydrophobic (Michel et al., 2001). Additionally, peat moss-based substrate leads to more nutrient leaching than bark substrate, which may be due to its higher content of macropores (>50 nm, 11%) comparing to bark substrate (7%) (Drzal et al., 1997).

2.2 Environmental concerns caused by peat moss

Harvesting peat moss for container substrate from peatland has interfered peatland's ecological functions (Leifeld and Menichetti, 2018). Peat moss harvesting reduced peatland C capacity, thus hindered its climate change mitigation capacity (Alexander et al., 2008). Also, harvesting peat moss disturbed N and CH_4 cycles (James et al., 2021). Additionally, peatland disturbance may bring challenges to the native animals, making it harder for them to find new habitats, thus reduce ecosystem biodiversity (Alexander et al., 2008).

Besides interfering with peatland's ecological functions, peat moss, as a container substrate component, also creates environmental concerns due to nutrient runoff as well as pesticide runoff (Michel et al., 2001; Kumar, 2017). In a common nursery production, a 15% leaching fraction was recommended to prevent the buildup of soluble salts in the container substrate (Cahn and Phillips, 2019). However, extensive irrigation, fertilizers, and pesticides were more often applied to containers to reduce the risk of crop failure (Savvas et al., 2013). Plants can only use 50% of nitrogenous fertilizers applied even under ideal conditions (Sönmez et al., 2008; Savci, 2012). The excessive nitrogen (N), phosphorus (P), and potassium (K) were lost through runoff, causing environmental concerns such as eutrophication, dead zones, and algal blooms (Power and Schepers, 1989; Zhu et al., 2004; Savci, 2012). Because of the low irrigation efficacy (80% of water runoff) in container production, highly soluble pesticides such as acephate, glyphosate, and mefenoxam are likely to dissolve and move with runoff water to a containment water body (Poudyal and Cregg, 2019). A 10-year survey of major streams and groundwater found that 97% of stream water and 61% of shallow groundwater near agricultural areas had one or more pesticides present (Stone et al., 2014).

2.3 Challenges of peat moss

Peat moss encounters production challenges as its volume and area have been largely reduced. The total volume and area of global peatlands have been decreased at a rate of 0.05% annually and by 10%~20% since 1800 owing to harvesting and land development (Temmink et al., 2017; Heck et al., 2021). Peat production was estimated to have decreased in 2019 in some peatland-rich countries (Temmink et al., 2017). Peatland area in Estonia has declined from 22% coverage of the country to only 5.5% for the past decade (Orru and Orru, 2006; Karofeld et al., 2020). In Ireland, around 84% of ombrotrophic peatlands (bogs) have been affected by peat extraction (Renou-Wilson et al., 2019). In Germany and Netherlands, 98% and 95% domestic peatland area have degraded due to the extensive peat moss harvesting (Barthelmes, 2016).

Peat moss also faces legislation challenges due to the implementation of peatland restoration projects and carbon neutral plans (Peng et al., 2018). Several European countries including Belarus, Ireland, and Sweden, were planning or implementing peatland restoration projects, reducing peat production across Europe in the future (Carlile and Coules, 2011). In Canada, among the total of 27, 615 ha peat moss production areas, more than 31% has been or is currently restored or reclaimed, with another 3% converted to other land-use (Shotyk, 1988). Also, the UK and Europe have legislated laws in order to protect the peatland from being over harvested (Alexander et al., 2008; Carlile and Coules, 2011). In 2019, Ireland announced its plan to stop all peat harvesting by 2028 (Brioche, 2020). In the same year, Finland announced its goal to become carbon neutral by 2035 by phasing out peat production (Brioche, 2020).

3 Biochar replacing peat moss as a container substrate

3.1 Biochar has suitable properties

Although biochar properties vary widely, many types of biochar could fall into the recommendation range either by itself or by combining with other components (Huang and Gu, 2019). Detailed biochar properties have been reported by Lan et al. (Huang and Gu, 2019), we summarized in Table 1 to compare several differences between biochar and peat moss-based substrates used in containers. For the most commonly used container substrate components such as peat moss and perlite, their total porosity was high, 83% and 92%, respectively, indicating low total porosity components need to be included to reach the ideal range (50-85%). As far as pH concerned, peat moss and vermicompost had a low pH lower than 5, 4.3-5 and 4.8, respectively, indicating that other alkaline components such as mixed hardwood biochar (pH 10.8-11.8) need to be incorporated to reach the ideal pH range (5.4-6.5) for container substrate (Table 1). For vermicompost and chicken manure, since their electricity conductivity and bulk density were high, 6.7 and 32.9 mS cm^{-1}, 0.38 and 0.62 g cm^{-3} respectively, their amount needs to be considered carefully when adding them into container substrates. Pinewood biochar, mixed hardwood biochar, and sugarcane bagasse biochar used in our previous studies had similar total porosity (74~85%), air space (3~34%), and bulk density (0.09~0.17 g cm^{-3}) to peat moss (83%, 19%, and 0.08 g cm^{-3}, respectively) and peat moss-based commercial substrate (71~78%, 3~20%, and 0.11 g cm^{-3}, respectively) (Guo et al., 2018b; Webber et al., 2018; Huang et al., 2019a; Yan et al., 2020; Yu et al., 2020b).

Unlike peat moss, which may encounter rewetting difficulties, certain types of biochar used in containers are easy to rewet due to its larger surface areas and pore size distribution (Lehmann et al, 2011). Biochar made from organic materials at 400 ~1,200°C, has larger surface area than peat moss because its higher micropores content (Lee et al., 2015). The surface area of biochar increased because high temperatures changed more macropores into mesopores/micropores in biochar (Lee et al., 2015). Micropores contributed largely to biochar surface area, endowing high adsorptive capabilities on the biochar and allowing small

TABLE 1 The physical properties including total porosity (TP, %), container capacity (CC, %), air space (AS, %), bulk density (BD, g cm-3), and particle size (PS, mm); chemical properties including pH, electrical conductivity (EC, mS cm-1), cation exchange capacity (CEC, meq 100g-1) and biological properties (microorganisms, MC) of several types of biochar and peat moss-based commercial substrate from our previous studies.

Properties	TP (%)	CC (%)	AS (%)	BD (g cm^{-3})	PS (mm)	pH	EC (mS cm^{-1})	CEC (meq 100g^{-1})	MC
Ideal Range	50~85	45~65	10~30	0.19~0.7	N/A	5.4~6.5	<0.75 (seedlings) <1.5(general crops)	6~15	N
PB	83	48.6	34.2	0.17	0.59~2	5.4	N/A	N/A	N
HB	85	60.3	24.4	0.15	67.3% >2	10.8~11.8	0.11	N/A	N
SBB	74	66~85	3~9	0.09~0.11	0.17(mean)	5.9	0.08	N/A	N
Peat moss	83	64	18.9	0.08	N/A	4.3-5	N/A	7~13	N
Perlite	92	59	34	0.05	N/A	7.3	0.01	~0	N
VC	75	72	3	0.38	89.4%<2	4.8	6.7	N/A	Y
CM	64	60	4	0.62	89.4%<2	7.5	32.9	N/A	Y
CS1	74~78	58~71	3~20	0.09~0.1	65.2%<2	N/A	N/A	N/A	N
CS2	71~75	84	15	0.11	N/A	6.8	0.07	N/A	N
PCS	79~97	47~85	12~31	0.15	3~6	6.5~6.75	0.18	N/A	N

Based on the studies from (Guo et al., 2018b; Huang et al., 2019a; Peng et al., 2018; Webber et al., 2018; Yan et al., 2020; Yu et al., 2020b). PB, pinewood biochar; HB, mixed hardwood biochar; SBB, sugarcane bagasse biochar; VC, vermicompost; CM, chicken manure; CS1, peat moss-based commercial substrate for plants growing; CS2, peat moss-based commercial substrate for plants propagation; PCS, pine bark-based commercial substrate; N/A, not applicable; N/Y in the microorganism column means mixes do not contain/contain microorganisms.

dimension molecules, such as gases and solvents to be absorbed (Lehmann et al, 2011). Thus, when the same irrigation practice applied, biochar would encounter less difficulties in rewetting than peat moss or peat moss-based substrate (Drzal et al., 1997).

3.2 Biochar has benefits on nutrients supply and absorption

Biochar was proposed to be beneficial to plant nutrient absorption because it could provide nutrient resources depending on its feedstock and production method. Lin et al. mentioned that acacia saligna biochar produced from at 380°C and sawdust at 450°C contained 17.7 and 16.2% of humics (humic-like and fluvic-like materials), which can serve as biostimulant and be assimilated by plants (Lin et al., 2012; Ding et al., 2016). Similarly, biochar made from gasified rice hulls at 815 ~ 871°C could be used as P and K fertilizers as the 5.4 g (0.19 oz) biochar sample released 35.2 mg (0.0012 oz) P and 50.1 mg (0.0018 oz) K in water solution for container crops over a short production cycle of 6 weeks (Altland and Locke, 2013). Pine bark biochar produced from 450°C fast pyrolysis increased mint growth due to its high K and P contents (Yan et al., 2020).

Also, biochar benefits plant nutrient due to its various properties. Adding green waste biochar to the substrate decreased the available N, resulting from biochar's porous structure induced N binding effects (Altland and Locke, 2012; Tian et al., 2012). Applying sugarcane bagasse biochar or mix hardwood biochar (pH 5.4 and 10.1 respectively) could adjust the substrate pH to around 6~8 (Yu et al., 2020b). The suitable substrate pH range (6~8) could promote K content, causing Mg and Ca deficiency due to the antagonism and/or synergism relationships among nutrients (Landis, 2005; Taiz and Zeiger, 2010).

3.3 Biochar effects on plant diseases

Soil-borne diseases affect potted plants' marketability and are hard to control (Katan, 1997; Graber et al., 2014; Puertolas et al., 2018). There are 10~20% of attainable crop yields loss caused by soil-borne diseases and the economic losses in USA are more than $4 billion (Graber et al., 2014). Soil-borne diseases control becomes more challenging due to trade globalization (Daughtrey and Benson, 2005; Puertolas et al., 2018). For instance, *Phytophthora ramorum* has survived for eight months in root balls and potting substrates of rhododendron plants, affecting the plants marketability worldwide (Appiah et al, 2004; Vercauteren et al., 2013). *Fusarium oxysporum f. sp papaveris*, a fungi pathogen attacking Papaveraceae plants, largely affected Papaveraceae plants marketability in Italy (Bertetti et al., 2018).

As a container substrate to replace peat moss, the effects of biochar on soil-borne pathogen has been less reported than that of plant growth, which had positive, neutral, and negative effects (Huang and Gu, 2019; Yu et al, 2020b). To date, there aren't enough studies about the biochar effect on plant health (Figure 2.), based on the Scientific Report database, among the 36,474 biochar publications (Figure 1.), only 3,997 were pathogen related, less than 11%. The majority of those pathogen studies were conducted in field, only 84 were conducted in containers. The dose of biochar is relatively low (ranging in most cases between 0.5~5%, Table 2) and most of the studies were conducted on edible crops such as tomato, pepper, strawberry, asparagus, lettuce, cucumber, beans etc (Copley et al., 2015; Mehari et al., 2015; Caroline et al., 2016; Jaiswal et al., 2017). The highest dose of biochar used in those studies is testing balsam fir bark and spruce bark biochar (475°C) on *Pythium ultimum* on sweet pepper, lettuce, basil, geranium and coriander at 50% (Gravel et al., 2013). Among those studies in Table 2, there were only two studies tested biochar effects on disease for ornamental crops which was red maple, red oak and geranium (Zwart and Kim, 2012; Gravel et al., 2013).

Similar to its effects on plant growth, biochar effects on plant health vary depending on plant species, biochar rates and types (Frenkel et al., 2017). Gravel et al. (2013) found that adding 50% of balsam fir/spruce bark biochar caused higher pathogen root colonization rate in all other crops except for coriander. Adding 30% coconut biochar increased plant health (Graber et al., 2014). Kadota and Niimi claimed that maple bark biochar improved the quality of several plant species, shortened the number of days needed for flowering, and increased plants survival rates (Kadota and Niimi, 2004). Adding 3% (w/w) wood-derived biochar with pre-conditioning such as pre-planting fertigation of the media reduced pre-emergence damping off caused by *Pythium aphanidermatum* by 71% for cucumber seedlings (Jaiswal et al., 2019). Incorporating biochar at rates of 10-30% (by vol.) increased strawberry fresh weight by 5-10% and reduced *Phytophthora* presents (Blok et al., 2019). Earthworm, microalgae biomass and 6% biochar mix increased tomato, pepper and eggplant seeds' resistance for *Pythium* sp., increased germination rate by 34% (Alshehrei et al., 2021). Adding 20% and 50% of mixed hardwood biochar decreased poinsettia root rot disease caused *Pythium aphanidermatum* and pepper blight disease caused by *Phytophthora capsica*, respectively (Yu et al., 2021; Yu et al. 2023).

The potential mechanisms on how biochar may influence plant disease include both direct and indirect influence on pathogen: 1) biochars' chemical compounds affect pathogen growth; 2) biochars' physicochemical properties improve soil nutrients availability and abiotic conditions; 3) biochars' physical properties help absorb toxins and enzymes produced by pathogens, reducing virulence; 4) biochars' presence induces systemic resistance in host plants; 5) biochars' physical properties enhance abundance and/or activities of beneficial microbes; 6) biochar induced disease suppression related gene expression (Graber et al., 2014; Bonanomi et al., 2015; Jaiswal et al., 2018; Jaiswal et al., 2020; Rasool et al., 2021; Ji et al., 2022; Liu et al., 2022).

4 Environmental benefits of biochar as a container substrate

4.1 Biochar protects peatland

The horticulture industry demands a large amount of peat moss as container substrates. Around 0.15 M m^3 of peat moss were used in container plants production, accounting for 86.5% of the total

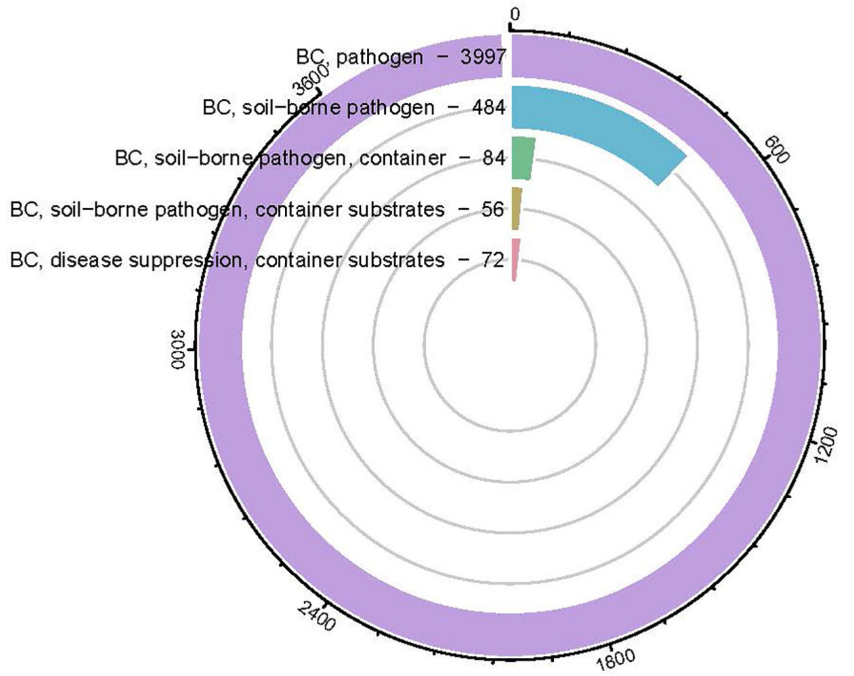

FIGURE 2
Circular bar-plot indicating the number of biochar (BC) pathogen-related articles published from 2010~2023 based on key words searching in Science Direct data base.

imported peat moss in the United States (USDA-NASS, 2018). In the United Kingdom, 0.06 M m^3 peat moss were used in horticulture, including container plants, bedding plants, vegetables, soft fruit, and cut flower production. In Europe, around 2.6 M m^3 peat moss were used in horticulture, with the total ratio of peat in media for plant growth being 99% in Estonia, 99% in Lithuania, 92% in Latvia, 88% in Finland, 87% in Ireland, 87% in Denmark, 87 in Sweden, and 81% in Germany (Kitir et al., 2018).

Replacing peat moss with biochar protects peatland from further disturbance. The highest rate for biochar replacing peat moss as a container substrate is 80% with pine bark biochar (Guo et al., 2018b; Huang et al., 2019b). If 80% of peat moss can be replaced by pine bark biochar, 0.12 M m^3, 0.05 M m^3 and 2.08 M m^3 peat moss can be saved annually in the United States, in the United Kingdom, and in Europe, respectively. Global average dry biomass Sphagnum production is around 260 g m^{-2} yr^{-1}, depending on species and locations (Gunnarsson, 2005). Considering the commercial peat moss bulk density is 0.1 g cm^{-3}, if 80% of peat moss substrate can be replaced by pine bark biochar, 46.2 M m^2, 19.2 M m^2, and 800 M m^2 of peatland can be saved annually from being disturbed for the United States, the United Kingdom, and Europe, respectively.

4.2 Biochar reduces chemical leaching

4.2.1 Biochar reduces nutrient leaching

As aforementioned, fertilizer tends to be over-used in greenhouse/nursery production and plants can only use 50% of fertilizers applied (Sönmez et al., 2008; Savci, 2012). The rest of the other half of fertilizers were either lost in evaporation and/or reactions with organic compounds (Savci, 2012). Moreover, since the majority of fertilizers haven't been absorbed by plants, they can reach ground water and contaminate ground water (Power and Schepers, 1989; Zhu et al., 2004).

Biochar replacing peat moss as a container substrate reduces nutrient runoff either by providing additional nutrient content or alternating substrates' properties. Adding 15-20% gasified rice hull biochar (815 ~871 °C) in a peat-based substrate reduced nutrients such as NH_4^+, NO_3^-, $H_2PO_4^-$, HPO_4^{2-}, and K^+ leaching as it provided sufficient potassium (K) amount for geranium and tomato plants growing in containers (Altland and Locke, 2017). Jahromi et al. (2018) found that switchgrass (1,000 °C) biochar-amended substrates reduced the total nutrients lost from hydrangea containers because biochar addition increased substrate water holding capacity. Altland and Locke (2013) demonstrated that adding 10% saw dust biochar to peat moss-based substrate increased nitrate and phosphate retention and subsequently reduced their leaching. Adding conifers wood biochar (500 °C) into container substrate for lavender production reduced K leaching as it increased K content of the growing substrates significantly (Fascella et al., 2020). Woodchip biochar (450~600 °C) decreased more extractable total N including NO_3-N than peat moss substrates with similar seedlings growth (Prasad et al., 2018). Similarly, adding forest wood biochar (700 °C) at 7.5% with additional fertilizer reduced NO_3-N, K and P leaching compared to the peat substrate. Adding fresh wood screening at 7.5% and 15% (500-600 °C) decreased NH_4-N and K leaching compared to the peat substrate under both 1-fold and 1.5-fold fertilizer conditions (Chrysargyris et al., 2019).

TABLE 2 Biochar effects on plant pathogens.

Host plants	Pathogen	Biochar feedstock	Biochar temperature °C	Biochar rate	Reference
bean	*Rhizoctonia solani*	eucalyptus wood, greenhouse wastes	350, 600	0,1%, 3% (w/w)	(Copley et al., 2015)
cucumber, tomato, lettuce, sweet pepper etc.	*Rhizoctonia solani*	maple bark biochar		0,1%,3%,5% (w/w)	(Elmer and Pignatello, 2011)
strawberry	*Botrytis cinerea, Colletotrichum acutatum and Podosphaera apahanis*	citrus wood (CW), greenhouse wastes (GWC)	GWC at 450	1% or 3% (w/w)	(Harel et al., 2012)
asparagus	*Fusarium oxysporum f.* sp. *asparagi; F. proliferatum (fusarium crown and root rot)*	hardwood dust charcoal	N/A	0,1.5%,3% (w/w)	(Jaiswal et al., 2015)
asparagus	*Fusarium oxysporum f.* sp. *asparagi (Fusarium root rot)*	coconut fiber charcoal	N/A	0,10%,30% (v/v)	(Graber et al., 2014)
tomato	*Ralstonia solanacearum (bacterial wilt)*	municipal bio-waste charcoal, wood charcoal	N/A	0, 20% and other not-specified concentrations (v/v)	(Nerome et al., 2005)
red oak and red maple	*Phytophthora cinnamomi and P. cactorum (stem canker)*	pine	Between 550 and 600	0, 5, 10 and 20% (v/v)	(Zwart and Kim, 2012)
sweet pepper, lettuce, basil, geranium and coriander	*Pythium ultimum*	balsam fir bark and spruce bark	475	50% (v/v)	(Gravel et al., 2013)
tomato	*Fusarium* spp.	eucalyptus wood pepper plant waste	350/600	0,0.5%,1%,3% (w/w)	(Jaiswal et al., 2017)
tomato pepper	*Botrytis cinereal* *Leveillula taurica*	citrus wood	N/A	1%,3%,5%(w/w)	(Elad et al., 2010)
lettuce	*OTC (antibiotic)*	bamboo	600	2%	(Duan et al., 2017)
cucumber	*Rhizoctonia solani*	eucalyptus wood and greenhouse wastes	350/600	0%~3%	(Jaiswal et al., 2014)
beans	*Rhizoctonia solani*	eucalyptus wood and greenhouse wastes	350/600	0%~3%	(Jaiswal et al., 2015)
rice	*Meloidogyne graminicola*	holm oak wood	650	0.6%, 1.2%, 2.5%, 5.0%	(Huang et al., 2015)
tomato	*Botrytis cinerea*	greenhouse wastes	450	0, 1, and 3% (w/w)	(Mehari et al., 2015)
lettuce strawberry	*Rhizoctonia solani* *Botrytis cinerea*	holm oak wood	650	0, 1, and 3% (w/w)	(Caroline et al., 2016)
carrot	*Pratylenchus penetrans*	pinewood, pine bark, wood pellets, spelt husks	500	0.80%, 0.92%, 1.24%, 0.64%	(George et al., 2016)
sweet pepper, tomato, lettuce, carrot, radish	*Rhizoctonia solani*	maple wood bark	700	0,1%,3%,5% (w/w)	(Copley et al., 2015)

N/A, Not applicable.

4.2.2 Biochar decreases pesticides usage and leaching

The over-use of pesticides in greenhouse production also caused environmental concerns (Ayoub, 1999; Bolognesi, 2003). In the United States, among the total usage of pesticide, around 90% of pesticide comes from agricultural production (Atwood and Paisley-Jones, 2017). Pesticides contaminate the environment via surface runoff, spray drift, and subsurface flow, which is the major pathway for pesticides entering water bodies (Zhang et al., 2018). Leaching can rapidly transport pesticides to surface and subsurface receiving waters (Roseth and Haarstad, 2010). The best management practices are recommended for nurseries to reduce pesticide contamination, yet, the best management practices alone may not completely remove pesticides contamination (Grant et al., 2019).

Biochar has been reported as a good sorbent for efficient removal of chemicals, and its efficacy depends on many factors including biochar types, effect of time, adsorbent dosage, chemical concentration and pH. Taha et al. (2014) demonstrated that biochar made from corn stover and rice straw adsorbed many types of pesticides including organophosphates (diazinon and malathion) and neonicotinoids (imidacloprid and acetamiprid). Mandal et al. (2017) reported that rice straw biochar had the highest adsorption rate for atrazine and imidacloprid. Baharum et al. (2020) found that activated coconut fiber biochar (700°C) removed 98.96% and 87.93% of diazinon respectively when modified with phosphorus acid and sodium hydroxide at pH 7. Ponnam et al. (2020) described that biochar produced from the neem tree bark (300°C) provided a 95.2% desirability on removal Bentazone with response (adsorption uptake) of 79.40 mg/g, for initial concentration of insecticide (50 mg/L), adsorbent dosage (0.448 g), time 30.0 min and pH 2. Gámiz et al. (2019) demonstrated that aged oak wood biochar (550° C) had a significantly higher removal rate (>85%) of three highly persistent and ionizable pesticides (imazamox, picloram, terbuthylazine) than the fresh biochar (<16%).

5 Economic benefits of biochar as a container substrate

Biochar provides large potential economic values as the market of biochar and biochar supply companies are growing. According to the transparency market research (Doe, 2014; Natural-Resources, 2017), the evaluated worth of global biochar market reached $0.44 M in 2016, and it is expected to experience a Compound Annual Growth Rate of 14.5% from 2017 to 2025 and reach a valuation of $1.48 M by 2025. Also, the number of biochar supply companies increased. There were approximately 150 biochar supply companies in 2013, mostly of them were small garden and specialty retailers, however, the number of biochar companies doubled in 2015 (Cedergreen et al. 2009; Jirka and Tomlinson, 2015).

5.1 Biochar decreases peatland restoration costs

Peatland restoration requires high economic costs such as techniques costs, rewetting and recurring costs, as well as maintenance costs (Glenk and Martin-Ortega, 2018; Humpenöder et al., 2020; Karofeld et al., 2020). The costs associated with restoration range from $280 ha^{-1} to $14,016 ha^{-1} (Moxey and Moran, 2014). A one-time cost of $7,000 ha^{-1} for initial rewetting and recurring was estimated, with another cost of $200 $\text{ha}^{-1}\ \text{yr}^{-1}$ maintenance and/or $140 $\text{ha}^{-1}\ \text{yr}^{-1}$ management costs (Glenk and Martin-Ortega, 2018).

Replacing peat moss with biochar as a container substrate largely reduces peatland restoration costs because biochar production does not degrade the peatland ecosystem. With around 10.3 M ha peatland area needs to be restored (Humpenöder et al., 2020), an estimated $72.1 billion one-time rewetting and recurring costs with another $2.06 billion and/or $1.44 billion maintenance and management costs could be saved annually by replacing peat moss with biochar.

5.2 Biochar reduces substrate costs

Replacing peat moss with biochar as a container substrate can bring large economic benefits due to its potential low price and large demand. The average customer price for sphagnum peat increased from $ 22 m^{-3} in 1986 to $172 m^{-3} in 2018 (Yu et al., 1990; Bwi, 2018). Customers may have to pay higher prices based on the distributors they chose, for instance, the price of peat moss in Greenhouse Megastore is $ 310.7 m^{-3} (Megastore, 2019). Comparing to peat moss, however, the average biochar price is $100 m^{-3}, half the price of peat moss from BWI, and one third the price of peat moss from Megastore. Aforementioned, 0.15 M m^{-3}, 0.057 M m^{-3}, and 2.6 M m^{-3} of peat moss were used in horticulture in the United States, United Kingdom, and Europe, respectively (Kitir et al., 2018; USDA-NASS, 2018). With 80% of biochar being able to replace peat moss as a container substrate (Guo et al., 2018b; Huang et al., 2019b), $8.64 M, $3.6 M, and $149.76 M can be saved annually in the United States, United Kingdom, and Europe, respectively if consumers get peat moss from a cheaper distributor. If consumers get peat moss from a more expensive distributor, $25.2 M, $10.5 M, $436.8 M can be saved annually in the United States, United Kingdom, and Europe, respectively. The actual economic benefits of using biochar to replace peat moss as a container substrate could be even larger if biochar were produced locally, which may lead to an even lower price than the average.

Also, using biochar to replace peat moss as a container substrate brings large economic benefits due to several reasons (Table 3). Firstly, peat moss needs a specific condition to growth such as waterlogged, acidic and anaerobic areas while biochar material can be grown anywhere. Secondly, peat moss regrowth rate ranges from 30-40% while biochar materials can reach to100%. Moreover, the price for commercially available peat moss is around $172 m^{-3}, if been purchased from wholesale such as BWI, 72% higher than that of biochar. Additionally, peat moss can only be harvested when the depth is more than 2m while biochar materials can be harvest or collected anytime. Peat requires thousands of years to be generated, making it a unrenewable resource (Hugron et al., 2013). With the restoration practices, the average rate of peat moss vertical growth was around 1 mm year^{-1} in the peatland (Savichev et al., 2020). If no restoration practices are launched, the spontaneous revegetation of abandoned peatlands will take even longer (Karofeld et al., 2020). The best suggested harvesting depth for peat moss is 0.25 m from the top soil, meaning after harvesting, peatland needs 25 years or even longer to be able to harvest again (Savichev et al., 2020). The 25 years are more than enough to grow pine trees to merchantable size for biochar production (Butler et al., 2017; Guo et al., 2018b). If we grow other biomass such as sugarcane (or other herbs), miscanthus, and shrubs, the generation of biochar can be 25 times faster than peat moss, providing 25 times the economic benefits of peat moss (Webber et al., 2018; Roy et al., 2020).

TABLE 3 The comparison between peat moss and biochar.

	Peat moss	Biochar
Source	Bog plants: moss, sedge…	Any biomass: sugarcane, bark, municipal wastes…
Formation	Plant material not fully decay	Chemical thermal reaction
Condition	Waterlogged, acidic, anaerobic	Oxygen-free, high temperature
Rate of regeneration	0.5~1mm year^{-1} (naturally)	Comparable to generation of biomass
Renewable	Yes	Yes
Regrowth	Yes, 30~40%	Yes, 100%
Main application	Fuel, soil amendments, potting mix	Fuel, soil amendments, potting mix, pollutant filtration
Price	~$172 m^{-3}	~$100 m^{-3}
Commercialization	Yes	Limited
Harvesting condition	Depth >2m	N/A
Reclaim rate	~25 yr (harvest wisely)	N/A
Restoration rate	1.5~10 cm year^{-1}	N/A

Information based on studies from (Yu et al., 1990; Bwi, 2018; Webber et al., 2018; Karofeld et al., 2020; Savichev et al., 2020). N/A means not applicable.

5.3 Biochar reduces chemical costs

Chemical costs in agriculture are high due to large demands and high prices. Global fertilizer demands were projected to 208 M tons with the United States consuming 22 M tons in 2015 at an average price $719 ton^{-1} (Baanante and I.F.P.R. Insitute, 1996; Schnitkey, 2017; EPA, 2019). Global pesticides use in agriculture was 4.12 M tons with USA using 408,000 tons, with the trade reached approximately 5.9 M tons valuing $37.6 billion in 2018 (FAO, 2020). The United States was the top five countries for pesticides imports with trade values ranging $1.4~3.0 billion in 2018 (Wanner et al, 2020).

Replacing peat moss with biochar as a container substrate significantly reduces chemical costs by adding extra nutrients, increasing nutrient use efficiency, and reducing disease incidence. Biochar produced from nutrient-rich raw materials could serve as a source of P and K, reducing the total amount of fertilizer needed for plant growth (Huang et al., 2019a). If using biochar could increase nutrient use efficiency by 50% (Jahromi et al., 2018), $7.91 billion can be saved in the United States, and $74.78 billion worldwide (assuming the average price was $719 ton^{-1}) (EPA, 2019). Also, mixed hardwood biochar used in our previous study could reduce 25% disease incidence, leading to less pesticide consumption (Unpublished Data). If using biochar could reduce pesticide usage by 25%, $9.4 billion could be saved globally.

5.4 Biochar decrease agricultural waste handling costs

Large amounts of agricultural waste contributed to high waste handling costs. Around 3.9 billion tons of waste were generated annually worldwide with 2.01 billion tons (expected to grow to 3.4 billion tons by 2050) being municipal solid waste (North America contributed 289 M tons) (Kaza et al, 2018). The operating costs for integrated municipal solid waste management, including collection, transport, treatment, and disposal, generally exceed $100 ton^{-1} yr^{-1} (USDA-EPA, 1997).

Using biochar to replace peat moss as a container substrate could significantly reduce agricultural waste handling costs. With pyrolysis for bio-oil purposes, the yield of biochar is from 20%~47% (Ok et al., 2015) (taking the average as 30%). To produce enough biochar for the horticulture industry in USA alone (0.15 M m^3), assuming all the wastes have similar density as municipal waste, 350 kg m^{-3}(USDA, 2008), nearly 0.18 M tons of agricultural waste can be converted, saving $18 M yr^{-1}. Similarly, to produce enough biochar for horticulture industry in United Kingdom (0.057 M m^3), and Europe (2.6 M m^3), 0.67 M tons, 3.03 M tons of agricultural waste can be converted, respectively, saving millions of dollars on agricultural waste handling.

6 Limitations and possible solutions for biochar as a container substrate

Using biochar as a replacement for peat moss as a container substrate provides many benefits, yet it has several limitations. Biochar limitations are mainly from the varied properties and potential toxic substances it may contain, the non-continuous biochar supply-demand loop, and the lack of awareness and production practice of using it as container substrates (Huang and Gu, 2019). Although the number of biochar literature has increased dramatically, there is still little awareness of biochar application among modern farmers (Wu et al., 2017). These limitations may be addressed by providing finically and nonfinancial policy support to motivate business practice change, improving biochar commercial availability, to educate consumers, extending biochar demand, and to establish good production and application practice, exploring more biochar application options (Pourhashem et al., 2019).

6.1 Biochar various properties and production

Unlike the well-established sphagnum peat moss, biochar properties vary widely depending on feedstocks, production temperature, and pre- and post-treatment, bringing application difficulties for consumers (Huang and Gu, 2019). Biochar may contain potential toxic compounds such as heavy metals, polycyclic aromatic hydrocarbons (PAHs) and dioxin depending on the raw material and producing conditions (Shackley et al., 2010). When incorporating biochar with heavy metals, PAHs and dioxin into container substrates, plant growth could be decreased.

Biochar's various properties could be addressed by implementing standard production practices such as using the same feedstock and temperature every time. Currently, most biochar is produced as a by-product from bio-oil-focused process, leading to various properties and toxic compounds (Huang and Gu, 2019; Yu et al., 2020a). Also, biochar made from feedstocks containing toxic compounds, either heavy metal, PAHs or chlorine could contain toxic compounds (Huang and Gu, 2019). As such, businesses can avoid producing toxic containing biochar by selecting feedstock material cautiously. Additionally, biochar various properties can be adjusted to an ideal range for container plants growth by incorporating other components such as bark, perlite, and peat (Guo et al., 2018b).

6.2 Biochar non-continuous supply-demand loop

Biochar supply and demand have not created a full loop for the industry yet. Consumers are reluctant to switch from peat moss to biochar due to their lack of awareness and poor biochar availability. Because of the unawareness of using biochar as container substrates, consumers tend to use the well-established and well-supplied peat moss as a major container substrate component, lowering biochar demand. In return, the low biochar demand discourages biochar producing companies due to the low financial benefits. Currently, there are only around 300 biochar companies worldwide, and most of them are small-scale companies, not being able to supply commercial biochar sustainably (Jirka and Tomlinson, 2015). Also, due to the lack of financial motivation, companies are not able to invest in biochar facilities, producing large-scale of container substrate-targeted/grade biochar (Pourhashem et al., 2019).

The non-continuous biochar supply-demand loop can be addressed by establishing related policies to encourage capital investment, providing technology support to reduce the initial production costs (Pourhashem et al., 2019). Academic world needs to pay more attention to the profitability of biochar application in their work (Maroušek et al., 2019). Also, non-financial programs, including extension programs can help educate consumers on biochar economic and environmental benefits and biochar application practices, increasing biochar demand. Additionally, more funding needs to be assigned to biochar research and development programs, exploring more biochar application options to enlarge biochar market margin.

7 Conclusions

As summarized in Figure 3, using biochar to replace peat moss as a container substrate for plant production provides an environmentally friendly way to address the environmental concerns associated with peatland mining and drainage, and additionally yields multiple benefits. Switching peat moss to biochar as a container substrate for plant production protects peatland ecosystem, increases water and fertilizer use efficiency, reduces greenhouse gas emission, and brings economic benefits. However, to reach biochar's full potential, biochar limitations such as the lack of awareness, potential toxic compounds, and the non-continuous supply-demand loop need to be addressed soon by

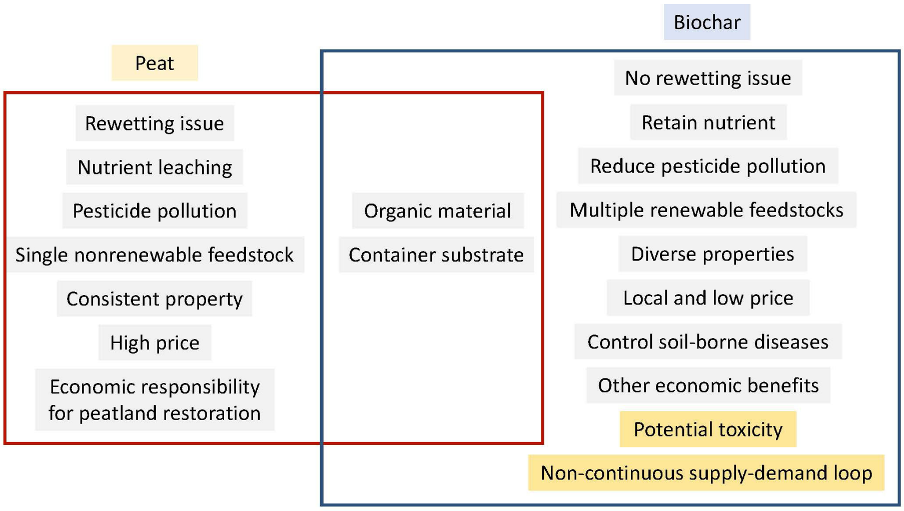

FIGURE 3
Figure of synthesis of peat moss and biochar comparison.

establishing both financial and non-financial supports from governments, companies, and research agencies.

Specifically, many container studies have been published on using biochar as an alternative for peat moss, however, most of the studies focused on crop production and the effect of biochar on disease control needs to be explored more. Studies testing the effect of the combination of bio-stimulants and biochar need to be explored for horticulture production. More molecular and physiology studies need to be included to enhance biochar application in horticulture. Also, nano-form of biochar products need to be developed and explored in horticulture. With many studies concentrated on edible crops, testing different biochar sources especially materials that may contain heavy metals such as sewage sledge and municipal waste is essential for safe food production. The facilities for biochar production needs high initial cost, preventing many companies from investing in biochar production, thus, appropriate technology for small to medium sized companies needs to be developed. Furthermore, the appropriate protocols that has been tested need to be shared to establish a uniform guideline for biochar production. Additionally, standardized biochar substrate mixes need to be commercialized for sustainable horticulture production. available, specifically for peat moss alternative growing substrate. In conclusion, using biochar in horticulture as a peat moss alternative can benefit environment economy significantly.

Author contributions

PY conducted the literature searching, collected, and analysed the data, and wrote the manuscript with the assistance of KQ, GN, and MG.

Conflict of interest

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

Publisher's note

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EDITED BY
Neftali Ochoa-Alejo,
Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico

REVIEWED BY
Sofia D. Carvalho,
Independent Researcher, Laramie, United States
Konstantinos Vlachonasios,
Aristotle University of Thessaloniki, Greece

*CORRESPONDENCE
Paul F. Devlin
✉ Paul.devlin@rhul.ac.uk

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Improved chilling tolerance in glasshouse-grown potted sweet basil by end-of-production, short-duration supplementary far red light

Firdous U. Begum[1], George Skinner[1], Sandra P. Smieszek[1], Simon Budge[2], Anthony D. Stead[1] and Paul F. Devlin[1]*

[1]Department of Biological Sciences, Royal Holloway University of London, Egham, United Kingdom,
[2]Vitacress Herbs, Chichester, United Kingdom

Sweet basil is a popular culinary herb used in many cuisines around the world and is widely grown commercially for retail as a live potted plant. However, basil is easily damaged by temperatures below 12 °C meaning plants must be transported from the grower to the retailer in a warm transport chain, adding considerable commercial cost in temperate countries. Improvement of chilling tolerance has been demonstrated in post-harvest crops such as tomato fruits and, indeed, fresh cut basil, by manipulation of the red:far red ratio of light provided to plants throughout the photoperiod and for a significant duration of the growing process in controlled environment chambers. We tested the effectiveness of periodic short-duration end-of-production supplementary far red light treatments designed for use with basil plants grown in a large scale commercial glasshouse for the live potted basil market. Four days of periodic, midday supplementary far red light given at end of production induced robust tolerance to 24 h of 4 °C cold treatment, resulting in greatly reduced visual damage, and reduced physiological markers of chilling injury including electrolyte leakage and reactive oxygen species accumulation. Antioxidant levels were also maintained at higher levels in live potted basil following this cold treatment. RNAseq-based analysis of gene expression changes associated with this response pointed to increased conversion of starch to soluble raffinose family oligosaccharide sugars; increased biosynthesis of anthocyanins and selected amino acids; inactivation of gibberellin signaling; and reduced expression of fatty acid desaturases, all previously associated with increased chilling tolerance in plants. Our findings offer an efficient, non-invasive approach to induce chilling tolerance in potted basil which is suitable for application in a large-scale commercial glasshouse.

KEYWORDS

sweet basil, cold tolerance, end-of-production, phytochrome, light supplementation, soluble sugars

Introduction

Sweet basil (*Ocimum basilicum* L.) is one of the most popular fresh herbs used throughout the world (Makri and Kintzios, 2008). As well as being a common ingredient in cooking, basil essential oils are commercially extracted for use as flavorings, fragrances, and additives in cosmetics and toiletries (Hiltunen and Holm, 1999). Their chemical composition is rich in linalool and methyl chavicol (Nacar and Tansi, 2000; Marotti et al., 2002). Basil essential oils have also been shown to have strong antimicrobial (Rattanachaikunsopon and Phumkhachorn, 2010) and antioxidant activity (van der Mheen et al., 2010), leading to suggestions of potential uses as a natural preservative (Takwa et al., 2018). As with many culinary herbs, basil is often purchased by consumers as a potted herb, making fresh material directly available from the kitchen windowsill, and this option is becoming increasingly popular. Over the last two decades sales of culinary potted herbs directly to consumers have increased dramatically. In the UK alone, in 2017, the industry produced over 20 million pots for supermarkets and sales of potted basil accounted for 40% of this market (Moncrieff, 2017). Basil is native to tropical and sub-tropical regions. As such, it is sensitive to chilling and temperatures below 12 °C cause strong wilting, discoloration of the leaves and loss of aroma (Fratianni et al., 2017). Fresh basil must, therefore, be maintained above this temperature in order to maintain commercial salability and this presents particular difficulties during distribution of fresh potted basil during winter months. Further issues for suppliers are created by the fact that the warm supply chain required for potted basil is counterproductive for more-temperate herbs such as coriander and parsley where shelf life is increased by maintenance at chilled temperatures (Cantwell and Reid, 1993; Loaiza and Cantwell, 1997). Potted herbs such as coriander and parsley are commonly transported alongside basil in mixed shipments meaning these ideal chilled temperatures cannot be used.

Post-harvest chilling damage during distribution and storage is a problem for a number of tropical and subtropical crops grown in greenhouses in countries with a temperate climate in the same way that it is for potted basil. Changes to membrane lipids resulting in loss of membrane integrity and increases in levels of reactive oxygen species in response to low temperature are two of the key primary effects leading to spoilage at chilling temperatures in many such cases (Sevillano et al., 2009). While temperate crops have mechanisms to adapt to low temperatures to prevent such damage, many tropical and subtropical crops lack the capability to adapt to the degree required and in time for protection mechanisms to be effective (Sevillano et al., 2009). Several studies have examined the induction of cold tolerance in fresh cut basil with a view to extending its shelf life by allowing it to be cold-stored. These treatments include pre- and post-harvest chilling or heat treatments. Conditioning plants using chilling temperature treatments prior to harvest has been shown to reduce damage. Specifically, it was demonstrated that a treatment of just 4 hours at 10 °C for 2 days prior to harvest would considerably extend shelf life of fresh cut basil stored at 5 °C but only if that 4 hour treatment was given at the end of the day (Lange and Camero, 1997). Conversely, Aharoni et al. (2010) demonstrated that exposure of freshly-cut basil shoots to 8 hours of humidified air at 38 oC, immediately prior to cold storage, dramatically reduced subsequent chilling damage. However, the same study suggested that this effect was due to it killing off spores of the spoilage fungus, *Botrytis cinerea* rather than any effect on the plant (Sharabani et al., 1999; Aharoni et al., 2010). Recently pre-harvest abscisic acid (ABA) application has also been found to reduce subsequent post-harvest chilling damage in fresh-cut basil (Satpute et al., 2019).

However, none of these treatments provide a viable approach for potted basil. While heat or cold treatment is viable for cut basil, large scale heat or cold treatments of potted basil would be expensive and technically demanding for the thousands of pots per day commonly supplied by growers. Spraying with ABA for either potted or cut basil is unlikely to be accepted with supermarkets. One study in tomato, however, provided an interesting additional possibility which could provide a very feasible approach to this problem. Wang et al. (2016) demonstrated that the light environment could have a significant impact on cold tolerance in seedlings of tomato. Plants have a wide array of photoreceptors that convey information about the light environment and allow plants to adapt their growth accordingly. Such photoreceptors control physiological and developmental process throughout the life history of the plant (Paik and Huq, 2019). The phytochrome family of photoreceptors detect red and far red wavelengths. They exist in two photo-interconvertible forms: an inactive, red-absorbing Pr form and an active, far red-absorbing Pfr form. Absorption of light converts Pr to Pfr and vice versa. This unique property of the phytochromes enables them to sense the reduction in the red: far red ratio (R:FR) in ambient light associated with vegetative shading. Light reflected from neighboring plants is specifically depleted in red light as a result of chlorophyll absorption resulting in a dramatic reduction in R:FR. This consequently leads to a depletion of the active Pfr form of phytochrome which triggers the shade avoidance syndrome, a response which includes a channeling of resources towards promotion of elongation growth (Franklin and Quail, 2010). Reduced R:FR has been associated with a wide range of changes in resilience in plants (Yang et al., 2016), not least, with increased freezing tolerance in Arabidopsis (Franklin and Whitelam, 2007). Following this observation, Wang et al. (2016) grew tomato plants in an enclosed controlled environment in monochromatic red light (R); monochromatic far red light (FR); or a mixture of monochromatic R and monochromatic FR in various proportions to generate different R:FR ratios throughout their 12 h photoperiod. They found that low R:FR ratios induced cold tolerance in tomato seedlings via mechanisms involving ABA and jasmonate (JA) signaling and increased expression of ABA and JA-regulated genes as well as induction of the C-REPEAT BINDING FACTOR (CBF) stress signaling pathway genes, which have previously been demonstrated to regulate cold resilience in plants (Thomashow, 2010). In a similar experiment, Larsen et al. (2023) very recently also found improved post-harvest chilling tolerance in fresh cut leaves from basil that had been grown in reduced R:FR ratio light throughout an 18 h photoperiod for one or three weeks prior to harvest in a vertical farming system. Larsen et al. (2023), however, found that ABA and JA were not altered in development of low R:FR-induced chilling tolerance in basil;

though, they observed increased levels of soluble sugars which they proposed may account for the improved chilling tolerance.

While such FR supplementation is manageable in a small-scale enclosed environment, the use of supplementary far red LED illumination throughout the photoperiod for one to three weeks in large scale, commercial glasshouse production would require considerable investment in further artificial lighting in order to illuminate a large proportion of the production area. Long term supplementary illumination would also add considerable extra energy costs. In addition, supplementary FR, generating R:FR ratios below 1.0 over such a period of time would cause considerable elongation growth in the crop, resulting in “leggy” plants. In a previous study, Larsen et al. (2020) showed that identical low R:FR ratio treatments to those which they later used to induce chilling tolerance also caused close to a 50% increase in plant height after three weeks of treatment. Reduction in leaf area was also seen following one week’s treatment. Unlike fresh-cut basil, such changes in morphology toward more “leggy” plants would not be a welcome trade-off for growers of potted basil, with retailers and customers associating a uniform, dense growth with healthy plants. Taller plants could also have the drawback of rendering pots unsuitable for current packaging.

Work by Fowler et al. (2005) demonstrated that induction of the cold response pathway in Arabidopsis, which confers freezing tolerance, is gated by the circadian clock, with the midday period being crucial for induction by cold treatment. Arabidopsis is tolerant of cold but requires a physiological priming, usually with cold treatment, in order to become tolerant of mild freezing conditions. Franklin and Whitelam (2007) demonstrated that treatment with low R:FR light can also trigger freezing tolerance in Arabidopsis. They found that simulated shade treatment upregulated the common CBF cold/drought responsive factor genes, indicating another example of crosstalk among stress responsive pathways. Building upon the work of Fowler et al. (2005); Franklin and Whitelam (2007) also demonstrated the key involvement of the circadian clock in gating the induction of the cold response pathway in Arabidopsis, meaning that mid-day FR supplementation alone was sufficient and induced the strongest response. They demonstrated that 4 h supplementary FR treatment around midday over a 4 day period was sufficient to induce freezing tolerance. Crucially, such mid-day 4h FR treatment would not induce significant shade avoidance phenotype (Cole et al., 2011).

Consequently, we sought to examine whether chilling tolerance could be induced in potted basil by 4 h supplementary FR treatment around midday. Also following the protocol of Franklin and Whitelam (2007), we sought to test whether 4 days of treatment at end of production was sufficient to induce this. In testing potted basil as opposed to fresh-cut basil, we also sought to examine whether the benefit of this supplementary FR could be maintained in living plants for sufficient duration to encompass transport to the retailer. We also sought to examine the effect of this four days of end-of-production, periodic midday supplementary FR on the transcriptome in order to better understand the impact at the molecular level.

We demonstrated that 4 h mid-day supplementary FR given over the final four days at end of production was able to confer greatly-improved chilling tolerance in potted sweet basil. Four hours of mid-day supplementary FR, below the minimal duration known to induce shade-related elongation growth (Cole et al., 2011), was applied periodically at midday, the time of maximal sensitivity for induction of cold tolerance pathways due to circadian gating (Kidokoro et al., 2009). Visible assessment of chilling injury and biochemical assessment of physiological markers of cellular damage demonstrated that, unlike control plants, FR treated plants sustained very little injury from 24 h incubation at 4 °C. Antioxidant levels, likewise remained high in FR-treated but not control plants following chilling. Transcriptomic analysis revealed changes in gene expression associated with a number of biochemical pathways previously linked with chilling tolerance. Increased expression of genes encoding enzymes involved in mobilization of starch into soluble sugars, particularly, raffinose and stachyose; synthesis of protective anthocyanins; and synthesis of amino acids was observed. We, therefore, demonstrate that basil’s tolerance towards chilling temperatures can be increased through changes in growing conditions that could feasibly be applied in a commercial glasshouse setting; thus, improving shelf life and reducing wastage, while also providing understanding of the impact of such changes within the plant at the molecular level.

Materials and methods

Plant material and growth conditions

Pot-grown sweet basil, *Ocimum basilicum* var. Marian (Enza Zaden, UK), were grown by Vitacress Herbs (Runcton, UK) in a commercial glasshouse. All pots used in this assay were collected in winter at 50 days old at which point they were ready for the market. Plants were grown in TPS peat substrate mix (Jiffy Products International, Moerdijk, The Netherlands). This contained 1 kg m^{-3} Tref Base fertilizer (final NPK+Mg: 17:10:14 + 4 plus trace elements, pH 5.8) and 0.25 ml m^{-3} FIBA-ZORB water holding agent (Turftech, Preston, UK) with 30 seeds per 0.4 L pot. Pots were maintained in a tightly packed arrangement in darkness for the first seven days. Pots were then spaced to 70 pots m^{-2} and the plants were grown under natural light, supplemented as necessary over a central 12 h period during the day by SON-T high pressure sodium lamps (140 µmol m^{-2} s^{-1} photosynthetically active radiation, PAR) when the natural light was less than 180 µmol m^{-2} s^{-1} PAR. This results in a photoperiod of 12 h in winter, stretching up to 18 h in summer. Average daylight PAR at the growing bench peaks at approximately 85 µmol m^{-2} s^{-1} at midday in winter up to 230 µmol m^{-2} s^{-1} at midday in summer, with additional shading is applied to the glasshouse in summer. Typical daily light integral (DLI) ranges from 8 mol m^{-2} d^{-1} in winter up to 12 mol m^{-2} d^{-1} in summer and this corresponds to a typical growth period of up to 52 days in winter, and approximately 32 days in summer. Plants were grown under controlled ambient conditions with an average temperature of 20.2 °C (± 0.8 °C) during the day and 18.3 °C (± 0.9) during the night. During the first 7 d, pots were watered with potable water as required to keep the substrate moist. Watering was carried out from the base, as required, by flooding pots 20 minutes at a depth of 3 cm then draining. After spacing they were then watered with water and/

or Tref Base fertilizer in order to keep the substrate moist while maintaining electrical conductivity between 2.2 to 2.8 µS cm^{-1}.

Pots were transferred from the Vitacress glasshouse to our experimental facility at the end of the 12 h photoperiod. Before transfer, pots were watered by flooding with potable water for 15 min. They were then placed in darkness for 12 h at 21 °C before transfer to the following experimental conditions. Control plants were maintained in 12 h white light/12 h dark cycles at 21 °C for four days. This provided a PAR of 50 µmol m^{-2} s^{-1} and a R:FR ratio of 5.11 (the ratio of intensity of 10 nm bandwidths centered around 660 nm and 730 nm). Experimental plants were maintained in the same 12 h white light/12 h dark conditions but were additionally treated with mid-day supplementary FR illumination from 4 h after dawn to 8 h after dawn, generating a R:FR ratio of 0.16. White light was provided by Osram Lumilux T5 HE 35 W/830 cool-white fluorescent tubes. FR illumination was provided by FR LEDs (Λ max 735 nm, Shinkoh Electronics, Tokyo, Japan). All light measurements were made using a StellarNet EPP2000-HR spectroradiometer (StellarNet Inc., Tampa, FL, USA). After 4 d, plants were transferred at 4 h after dawn to either 21 °C or 4 °C for 24 h. This final incubation was carried out in dim light conditions (5 µmol m^{-2} s^{-1}, Osram Lumilux T5 HE 35 W/830 cool-white fluorescent tubes) to simulate typical lighting conditions during commercial transport and storage.

Visual assessment of chilling damage

A scale for the grading of the basil pots exposed to chilling temperature was based on preliminary observations which demonstrated that chilling damage was first observed in the form of leaf wilting followed by discoloration of the leaves. A scale ranging from 1 to 5 was devised, where 1 represented no visible damage; 2 was scored if some plants within a pot exhibited only slightly wilted leaves; 3 was scored where some plants within a pot exhibited heavily wilted leaves; 4 was scored if some plants within a pot exhibited wilted leaves and discoloration was observed on less than 50% of the leaves in the pot; and 5 scored if some plants within a pot exhibited wilted leaves and discoloration was observed on more than 50% of the leaves in the pot. Representative pictures of pots exhibiting each level in the grading scale are shown in Supplementary Figure 1.

Measurement of electrolyte leakage

The electrolyte leakage assay was modified from the methods described by Campos et al. (2003) and Bajji et al. (2002). At the end of the environmental treatment period, the six top-most fully expanded leaves from the basil plants in a single pot were removed to form each replicate and immediately immersed in 100 ml of distilled water in a crystallizing dish (150 ml). The leaves within the crystallizing dish were separated from one another by a nylon mesh. The crystallizing dishes were then incubated at 21 °C in darkness on an orbital shaker at 40 RPM. The electrical conductivity (EC) of the water in microSiemens (µS) was measured to obtain initial EC (ECi) and EC of the water containing leaf samples was measured after 12 h of incubation to obtain a final EC (ECf). The water and leaves was then autoclaved at 120 °C and 15 psi for 30 min, and another EC reading was taken to obtain total EC (ECt). Relative electrolyte leakage (EL) was calculated using the formula EL (%) = (ECf - ECi)/(ECt - ECi) x 100.

DAB (3,3′-diaminobenzidine) staining

The DAB assay was carried out according to the protocol described by Daudi and O'Brien (2012). To prepare the DAB staining solution, 0.1% DAB was prepared with sterile H_2O, with the pH being reduced to 3.0 with 1M HCl to allow dissolution of the DAB. Tween 20 was then added to 0.05% (v/v) and Na_2HPO_4 to a final concentration of 10 mM. The three top-most fully expanded basil leaves per replicate pot were removed and placed within a sterile Petri dish then immersed in 25 ml of the DAB staining solution. Leaves inside the Petri dishes were vacuum infiltrated using a gentle vacuum for 5 min then the dishes were covered with aluminum foil and incubated at 30 °C for 4-5 h on an orbital shaker at 55 RPM. Following incubation, the DAB staining solution was removed and replaced by a bleaching solution (ethanol: acetic acid: glycerol = 3:1:1) in order to bleach chlorophyll. The plates were incubated for 15 min at 95 °C then the bleaching solution was removed and replaced by fresh bleaching solution before incubation for a further 30 min at 95 °C. Following this, the leaves were rinsed with water and then photographed under uniform lighting. Relative H_2O_2 accumulation was calculated using ImageJ (Schneider et al., 2012), using the color threshold tool to measure the area of a leaf covered by the brown precipitate and expressing this as a percentage of the area of the entire leaf.

FRAP assay

Water soluble antioxidants were assayed using the ferric reducing ability of plasma (FRAP) assay as described by Benzie and Strain (1996). The top-most fully expanded single leaves were weighed then flash frozen in liquid nitrogen in 1.5 ml microcentrifuge tubes and stored at -80 °C until assayed. For the FRAP assay, these leaves were ground to a fine powder in a pestle and mortar with sand. After grinding, 0.6 ml of acetate buffer (300 mM, pH 7.6) was added to the mortar and the lysate was then transferred to a new 1.5 ml microcentrifuge tube. A further 0.6 ml of acetate buffer was added to the mortar to recover the remaining lysate which was also added to the same microcentrifuge tube. The lysate was then centrifuged at 15,000 g for 4 min and the supernatant, containing the water-soluble antioxidants, was transferred into a clean 1.5 ml microcentrifuge tube.

Antioxidant activity was compared to that of ascorbic acid standards using freshly prepared FRAP solution (25 ml of 300 mM acetate buffer pH 3.6, 2.5 ml of 20 mM Ferric chloride hexahydrate and 2.5 ml of 10 mM 2,4,6-Tripyridyl-s-Triazine in 40 mM HCl). To 30 µl of the sample or standard, 300 µl of FRAP solution was added. The intensity of the colored product resulting from reduction of Fe^{3+} ions to Fe^{2+} was determined by measuring absorbance at 590 nm using a SpectraMax Plus 384 microtiter plate spectrophotometer (Molecular Devices, Wokingham, UK). Ascorbic acid standard curves with correlation coefficients of not less than 0.99 were used to quantify the antioxidant activity in the experimental samples and antioxidant activities were then normalized by fresh weight.

Extraction of total RNA from basil tissues

Total RNA was extracted from leaf tissues of basil using an RNAeasy plant mini kit (Qiagen, Manchester, UK). Total RNA was extracted from the top-most fully-expanded single leaves from at least five biological replicates for each environmental condition and was pooled into one sample for each condition. RNA integrity and purity were checked by carrying out RNA gel electrophoresis, and by measuring the absorbance at 260 nm, 280 nm and 230 nm using a Nanodrop spectrophotometer ND-1000 (Qiagen, Manchester, UK).

RNA sequencing

All samples were sequenced at the sequencing core facility, Cleveland Clinic, Cleveland Ohio. Prior to sequencing, sample quantity and quality was measured using a Qubit fluorometer (Thermo Fisher Scientific – Waltham, MA, USA). The RNA sequencing library was prepared using the protocol described by (Wang et al., 2011). Briefly, mRNA was obtained from the total RNA pool before fragmentation, cDNA synthesis, adapter ligation and size selection; and the size-selected ligated DNA products were then amplified by PCR to produce a sequence-ready library. The cDNA was then subjected to paired-end high-throughput sequencing using an Illumina Hiseq2500 (San Diego, CA, USA).

Differential expression analysis

Analysis of RNAseq data was carried out via the Galaxy platform (Afgan et al., 2022). Quality of raw sequence data was assessed through the FastQC package (Andrews, 2010). Adapter sequences were removed with the Cutadapt package (Martin, 2011) and sequences with a low-quality score were processed with the Trimmomatic package using default settings (Bolger et al., 2014). A *De Novo* transcriptome assembly was constructed using the Trinity 2.2.0 package (Haas et al., 2013) with paired reads used as input. Forward and reverse read trimmed sequence files were pairwise aligned to the Trinity assembled transcripts using the HISAT2 2.1.0 package (Kim et al., 2015). Aligned reads were then assembled using StringTie 1.3.4 (Pertea et al., 2015). The StringTie assembled transcripts for the various samples were then combined using StringTie merge 1.3.4 (Pertea et al., 2015) into a single non-redundant list that was used as a global set for reference for differential expression analysis between the samples. GFFCompare 0.9.8 (Pertea et al., 2020) was then used to compare the StringTie merge global set to the Trinity assembled transcripts in order to annotate transcripts with respective Trinity ID codes. Gene expression was then measured using the featureCounts 1.6.3 package (Liao et al., 2014). Annotated transcripts from GFFCompare were input with aligned transcripts from the HISAT2 package to output a table containing counted fragments per gene. Data from featureCounts was then used to produce a table of normalized FPKM counts for the comparison of gene expression across the tested samples.

In order to carry out functional analysis, the Trinity assembly file was annotated with gene identifiers using the NCBI BLAST+ BLASTx command line application, using a reference database created from the Uniprot Arabidopsis proteome (uniprot.org, ID: UP000006548). Arabidopsis Genome Initiative (AGI) TAIR10 ID annotations were applied where the p-value of the top match was below $1x10^{-4}$ and the bit score above 50. Where multiple fragments were mapped to the same AGI ID, their expression was averaged per AGI ID. The Panther functional classification tool (Mi et al., 2019) was used to assign transcriptome-wide biological process GO tags.

For relative expression analysis a minimum expression cut-off of 15 on average across the four samples for each AGI ID was first applied to the data. Relative change in expression for each AGI ID for the comparison between control and FR treated samples at each timepoint was then expressed as a log base 2 value. The PageMan 0.12 utility (Usadel et al., 2006) using AGI TAIR10 mappings was then used to assess overrepresentation of biological process gene ontology categories among differentially expressed genes, applying the default parameters. Z-scores for over- or under-represented biological processes were then represented using the conditional formatting function in Microsoft Excel. Highly differentially expressed genes were also assessed for over-representation of GO-Slim Biological Process tags via the Panther enrichment analysis tool using the Fisher's Exact test (Mi et al., 2019).

qRT-PCR

Reverse transcription of extracted RNA was performed with the QuantiTect Reverse Transcription Kit (Qiagen, Manchester, UK). gDNA was removed as per instructions, then 1 µg of RNA per sample was reverse transcribed. The cDNA synthesis was performed in a Techne 5-prime thermocycler (Cole-Parmer Ltd., Saint Neots, UK).

The primers used for assessing expression gene by qPCR were designed based on the Trinity-assembled transcripts, with the exception of *O. basilicum* Ubiquitin primers which were designed based on a published sequence obtained from GenBank: LN999820.1. The primer pairs were designed (150 bp maximum product length, optimal Tm at 60°C, GC % between 45 and 65%) using Primer3 software (Untergasser et al., 2012), favoring the 3' end of transcript sequences. Primer sequences are shown in Supplementary Table 1. In order to determine the specificity for the target sequence, standard PCR from cDNA was first carried out followed by sequencing of the product. PCR was performed using PCR BioMix^{TM} Red PCR reaction mixture (Bioline, London, UK). To confirm that the PCR was successful, 5 µl of each PCR product was run on a 3% TBE (Tris-borate-EDTA) agarose gel alongside a Biolone HyperLadder^{TM} 50 bp DNA ladder (Bioline, London, UK) to check for bands of the expected size. To be certain the primers were amplifying the target genes of interest, the gel-purified PCR products were sent to Eurofins Genomics (Wolverhampton, UK) for DNA sequencing. 25 µL of PCR product was run on a 2% TBE agarose gel. The bands were then cut out and purified as per the Wizard$^{®}$ SV Gel and PCR Clean-Up System (Promega, Southampton, UK) centrifugation protocol. 2 µl of loading dye (Bioline, London, UK) was mixed with 3 µl of the DNA extracted from the clean-up and run on a 2% TBE agarose gel to check the band intensities for DNA concentration. The cleaned DNA was then diluted and mixed with the necessary primers as per the Eurofins Genomics Mix2Seq protocol.

qPCR was performed in a Rotorgene 6000 thermocycler (Qiagen, Manchester, UK) using the primer sequences shown in Supplementary Table 1. *O. basilicum* Ubiquitin was used as the housekeeping gene. Three biological replicates and two technical replicates were used for each treatment sample. Samples were prepared with 0.5 µg of cDNA in a total volume of 20 µl, using the QuantiTect SYBR Green PCR Kit (Qiagen, Manchester, UK) with each primer added to a final concentration of 200 nM. A QIAgility automated pipetting robot (Qiagen, Manchester, UK) was used to prepare reactions in order to maximize pipetting accuracy. The following temperature program was used for qPCR: an initial denaturing of 94 °C for 2 min, followed by a cycle of 94 °C for 15 s, 60 °C for 45 s and 72 °C s for 30 cycles. Following qRT-PCR, melting curves for the products were obtained using the Rotor-Gene Q (ver.2.3.1.49) software (Qiagen, Manchester, UK) in order to confirm amplification of a single target. Ct values for the qPCR data were then obtained using the Rotor-Gene Q software and used to calculate relative expression using the ΔΔCt method (Rao et al., 2013).

Results

Daily, mid-day supplementary FR at end of production reduces appearance of chilling injury in greenhouse-grown potted basil

Greenhouse-grown potted sweet basil plants at a stage ready for market were transferred at dusk to a controlled environment undergoing 12 h white light: 12 h dark cycles at 21 °C. They were then either maintained in these control conditions or given a daily treatment of 4 h supplementary FR, generating a R:FR ratio of 0.16, beginning four hours (4 h) after dawn and spanning the middle of the day for four consecutive days. Plants from each treatment group were then either transferred to dim light at 21 °C or to dim light at 4 °C for 24 h to simulate low temperature transport and storage conditions. Damage due to chilling was then scored using a range of metrics. All plants which had been transferred to chilling conditions showed some degree of chilling injury, exhibited as wilting and leaf discoloration (Figures 1A, B). However, plants which had received supplementary FR treatment prior to transfer showed greatly reduced visible chilling injury following 4 °C treatment compared to untreated plants. While the majority of untreated plants showed both wilting and extensive discoloration, the majority of FR-treated plants within each pot showed no wilting and only minor discoloration (Figures 1A, B). Quantification of chilling injury using a scale assessing both the occurrence of wilting and the degree of discoloration, revealed a significant difference between control and FR-treated plants following cold treatment (Figure 1C). FR treatment, however, caused no visible difference in plants maintained in warm 21 °C conditions, notably confirming that, as expected, the plants showed no elongation growth or other morphological shade avoidance response to the mid-day FR treatment.

Markers of cellular damage and stress reveal a strong cold-protective effect of FR treatment

Electrolyte leakage from leaves was used as a proxy for cellular damage following cold treatment. Only minimal electrolyte leakage was observed in leaves from plants maintained in warm conditions and daily mid-day FR treatment had no effect on this (Figure 2). Cold treatment resulted in a significant increase in electrolyte leakage in leaves of both control and FR treated plants. However, levels of electrolyte leakage following cold were greatly reduced in plants that had previously been treated with FR compared to plants that had previously been maintained in control conditions (Figure 2). Thus, FR pre-treatment appeared to enable the plants to withstand much of the damaging effect of cold on the membranes of leaf cells.

Colorimetric assessment of Reactive Oxygen Species (ROS) accumulation was also carried out as a marker of plant stress at the cellular level. A DAB (3,3′-Diaminobenzidine) assay of whole leaves for the detection of H_2O_2 revealed that the leaves of plants which had been incubated at 4 °C contained significantly higher levels of H_2O_2 than those maintained in 21 °C. However, while high H_2O_2 levels were observed in both control and FR-treated basil leaves following cold, leaves of plants previously treated with 4 h mid-day supplementary FR for four days displayed much lower levels of H_2O_2 accumulation than those of untreated plants (Figure 3A). Quantification of percentage of leaf area revealing H_2O_2 accumulation demonstrated that FR-treated plants showed 58% lower H_2O_2 levels than untreated plants following cold exposure, indicating a significant difference in the degree of stress between FR-treated and untreated plants. Curiously, however, DAB staining did also reveal a small but significant increase in H_2O_2 accumulation as a result of the daily FR pulses in plants maintained in warm 21 °C temperature (Figure 3B) suggesting that this treatment did trigger a mild stress response in itself.

Crucially, though, the above assays for cellular damage and redox stress both confirmed a cold-protective effect of daily, mid-day supplementary FR.

Midday supplementary FR treatment reduces loss of antioxidants following chilling

A FRAP assay measuring water soluble antioxidant content revealed that chilling resulted in a strong reduction in antioxidant content in basil plants. Although the FR treatment did not prevent a

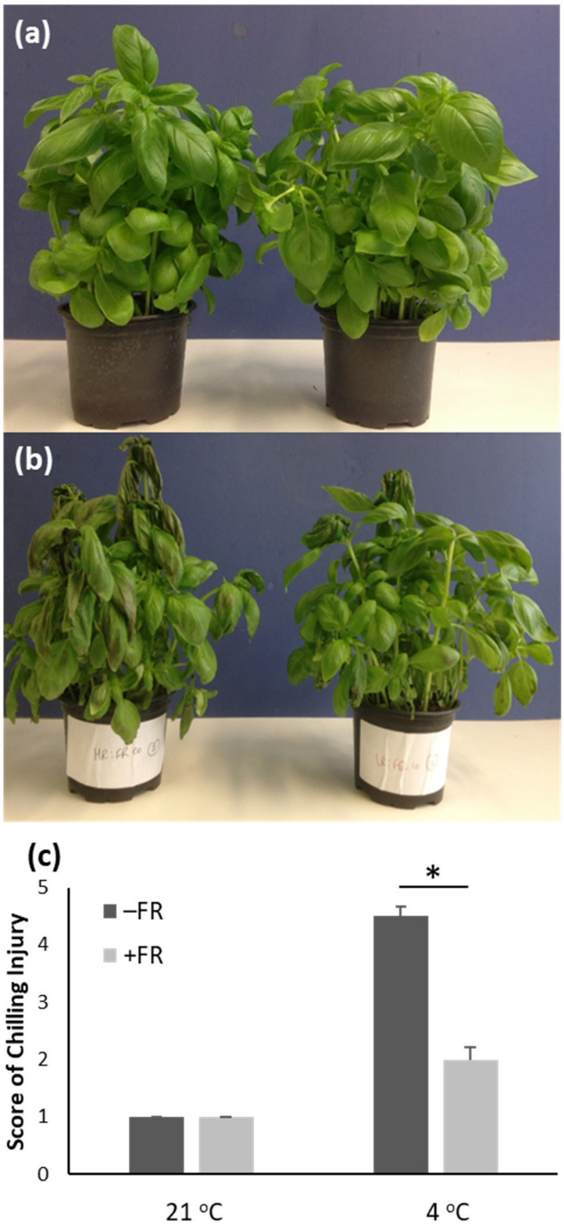


FIGURE 1
End-of-production midday supplementary FR treatment reduces chilling injury in potted basil. Glasshouse-grown market-ready basil plants were maintained at 21 °C in 12 hr light/12 h dark cycles either with 4 h supplementary FR in the middle of the day (right in images a and b) or without supplementary FR (left in images a and b) for 4 days. Plants were then either maintained in 21 °C **(A)** or transferred to 4 °C for 24 h **(B)**. Images show representative pots. **(C)** Mean chilling injury score based on phenotypic assessment for plants treated as above. Values represent mean ± SE for a minimum of seven pots. Asterisk represents significant difference, $p \leq 0.05$.

reduction in antioxidant content, the reduction was much less in FR treated plants than in control (Figure 4). Following cold treatment, water soluble antioxidant levels in control plants fell to 15% of those observed in plants maintained in warm conditions (an 85% reduction) whereas antioxidant levels in FR pre-treated plants fell to 40% (a 60% reduction). Thus, in addition to reducing damage at both a whole plant and cellular level, daily mid-day FR pre-treatment also helped maintain beneficial antioxidant levels in potted basil during chilling.

Global gene expression analysis reveals extensive transcriptomic reprogramming by midday FR

In order to determine the possible mechanism of action of end-of-production daily mid-day FR treatment in conferring subsequent cold tolerance in potted basil, we carried out RNA sequencing on FR-treated plants. Samples were taken from basil plants at the midpoint of the fourth and final mid-day FR treatment and two

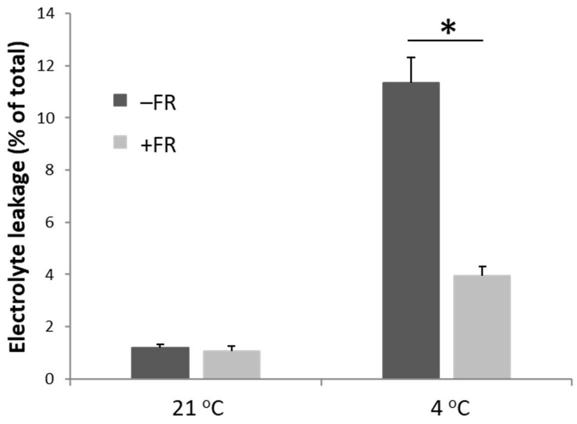


FIGURE 2
End-of-production midday supplementary FR pre-treatment reduces electrolyte leakage from leaves following chilling. Electrolyte leakage from leaves was measured in leaves of basil either maintained in control conditions or treated with 4 h midday supplementary FR for four days at end of production. Plants were then either maintained in 21 °C or transferred to 4 °C for 24 h prior to measurement. Data are mean ± SE for a minimum of 10 plants. Asterisk represents significant difference, $p \leq 0.05$.

hours post treatment in order to examine both acute and sustained changes in gene expression. The R:FR ratio of 0.16 generated by supplementary FR would be expected to trigger changes in gene expression associated with shade avoidance during the treatment (Devlin et al., 2003). However, following removal of FR, the R:FR ratio returned to 5.11, which would immediately return the phytochrome photoequilibrium to that found in an unshaded environment and reverse any changes in directly phytochrome-regulated gene expression (Roig-Villanova et al., 2006), allowing more-prolonged downstream effects to be identified. In the absence of a high-quality complete reference genome for the complex tetraploid genome of sweet basil (Gonda et al., 2020), we used *De Novo* assembly to reconstruct the transcriptome for the purpose of identifying biological processes impacted by mid-day FR treatment.

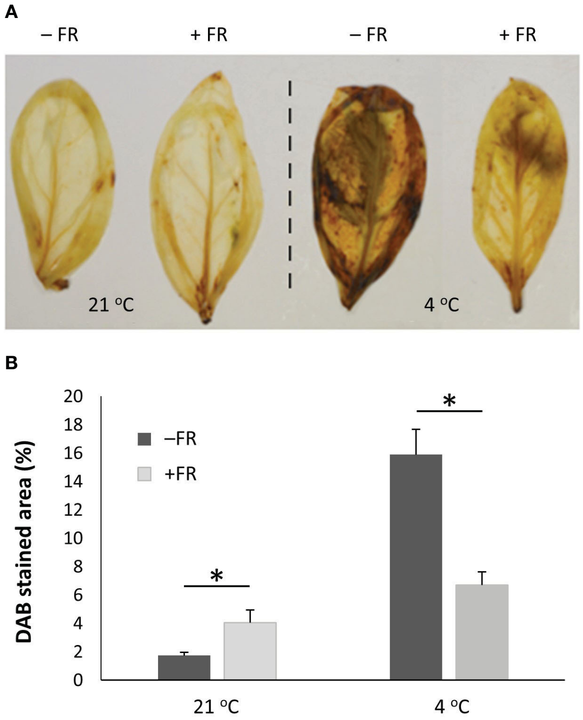


FIGURE 3
End-of-production midday supplementary FR pre-treatment reduces ROS accumulation in leaves following chilling. Plants were either maintained in control conditions or treated with 4 h midday supplementary FR for four days at end of production. Plants were then either maintained in 21 °C or transferred to 4 °C for 24 h **(A)** Representative leaves from a subsequent DAB staining assay revealing accumulation of hydrogen peroxide in basil leaves. **(B)** Percentage of leaf area showing DAB staining. Data are mean ± SE for a minimum of 15 leaves. Asterisks represent significant differences, $p \leq 0.05$.

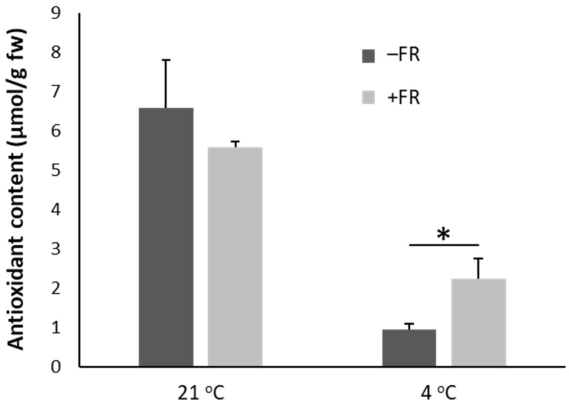


FIGURE 4
End-of-production midday supplementary FR pre-treatment reduces loss of antioxidants in leaves following chilling. Content of water-soluble antioxidants (equivalent to ascorbic acid, μmol/g fresh weight of leaf material) in leaves of basil either maintained in control conditions or treated with 4 h midday supplementary FR for four days at end of production. Plants were then either maintained in 21 °C or transferred to 4 °C for 24 h. Data are mean ± SE for a minimum of 10 plants. Asterisk represents significant difference, $p \leq 0.05$.

Following *De Novo* assembly of the transcriptome, gene expression levels were estimated by mapping clean sequence fragments to the assembled transcriptome. The abundance of the transcripts was normalized using the FPKM method. In order to allow later gene ontology analysis, the transcripts were then blasted against a reference database created from the Uniprot Arabidopsis proteome in order to map them onto closely related Arabidopsis proteins. Arabidopsis was chosen as a target due to the far more extensive functional designation of its proteome compared to that of basil or more closely-related fully-sequenced species for which little ontological information is available. The Blast-x tool, identified transcripts corresponding to 9,728 different orthologues of Arabidopsis proteins within the basil RNAseq transcriptome (Supplementary Table 2).

A global correlation analysis showed a high degree of correlation between the mapped transcriptomes of control versus FR treated plants; however, a number of genes showed greater than two-fold up or down regulation during or after FR treatment (Supplementary Figure 2). Mapped genes showing at least two-fold change in expression either during, or 2 h after, supplementary FR treatment were selected and assigned to groups based on their pattern of their response across the two time points. 671 genes showed at least a two-fold upregulation in expression during but not after FR treatment (no more than a 1.5-fold upregulation after FR treatment). 288 genes showed at least a two-fold upregulation both during and after supplementary FR treatment, while 300 showed at least a two-fold upregulation after supplementary FR but not during FR treatment (Figure 5). The lower number of genes showing persistent upregulation is consistent with the removal of the stimulus prior to the second time point; however, it is clear that there is a lasting effect of the treatment. In terms of downregulated genes, 243 genes showed at least a two-fold downregulation in expression in response to FR during but not after the FR treatment (no more than a 1.5-fold downregulation after FR treatment). 40 genes showed at least a two-fold downregulation both during and after supplemental FR, while a further 151 showed at least a two-fold downregulation after supplemental FR with no congruent change during FR treatment (Figure 5). Again, although fewer genes showed a persistent pattern of regulation, there is a clear prolonged effect of the FR treatment on a substantial number of genes, consistent with the observed ongoing effect observed for chilling tolerance.

Periodic midday FR treatment enhances expression of soluble sugar, selected amino acid, and anthocyanin biosynthetic pathways

In order to examine the likely long term physiological effects on the plant, we next undertook a gene ontology (GO) analysis. An initial genome-wide GO analysis of biological processes based on all mapped transcripts revealed that 79% of these transcripts fell within the parent categories, “cellular process”, “metabolic process”, “biological regulation”, “localization”, “response to stimulus”, and “signaling” (Supplementary Figure 3). However, to analyze biological processes enriched among genes identified as up or downregulated during and/or after FR supplementation, an enrichment analysis was then performed using more detailed classifications. A strong enrichment of photosynthesis-related terms was observed among genes temporarily downregulated during FR treatment. This includes genes associated with both photosystems I and II, and the Calvin Cycle. This was complemented by an underrepresentation of genes in this functional category among genes temporarily upregulated during FR. However, in both cases, this effect was no longer observed 2 h after FR treatment had ceased (Figure 6). At the same time, there was a strong overrepresentation of genes identified with gluconeogenesis and the TCA cycle among genes temporarily upregulated during but not after FR treatment. Several other enriched parent GO terms were also identified but almost all of those showed enrichment among genes showing persistent differential expression both during and after FR treatment. This includes extensive enrichment of child terms of the

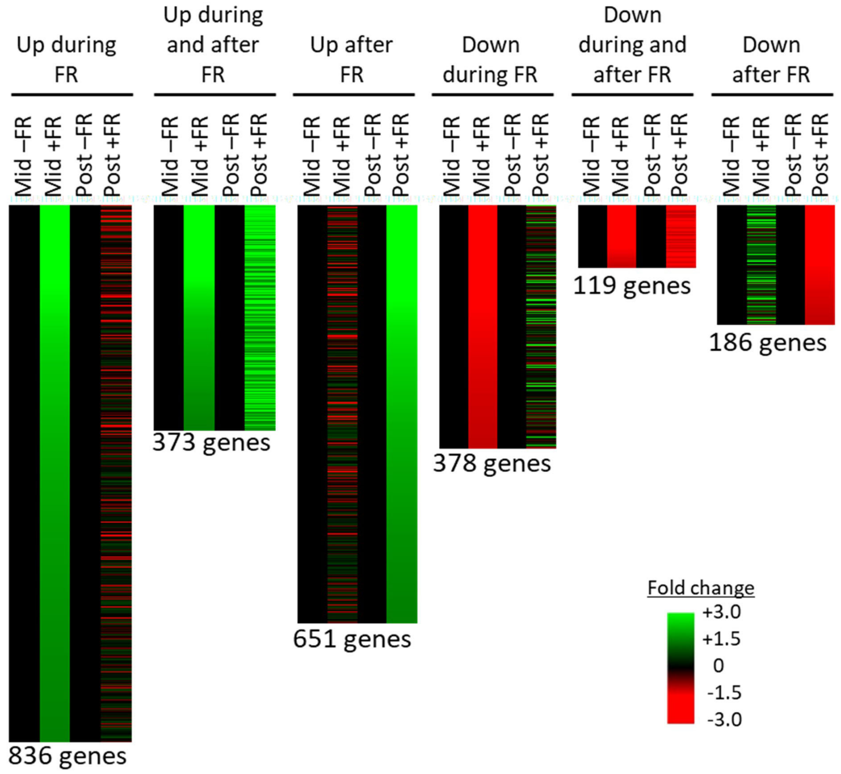


FIGURE 5
RNAseq reveals persistent changes in gene expression in basil as a result of end-of-production midday supplementary FR treatment. Transcripts identified by RNAseq showing at least two-fold change in abundance during (Mid) and/or 2 h after (Post) midday supplementary FR treatment. Transcripts are grouped according to pattern of response. Each transcript is represented by a separate line and fold change in treated (+FR) versus control plants (−FR) is shown for each time of sampling. Number of transcripts (genes) in each group is shown below each pattern.

major carbohydrate metabolism (major CHO metabolism) parent GO term, particularly starch and sucrose metabolism, with starch metabolism being overrepresented among upregulated genes both during and after FR treatment and sucrose metabolism being overrepresented among upregulated genes during FR treatment. By contrast, myoinositol metabolism was enriched among persistently-downregulated genes. The analysis suggested that cell wall metabolism is downregulated in a persistent way, with terms including both cellulose synthesis and cell wall degradation, cell wall proteins, and pectin esterases enriched among genes downregulated both during and after FR treatment. Similarly, lipid metabolism was indicated as being generally downregulated with fatty acid synthesis and lipid degradation terms being enriched among persistently downregulated genes. Notably, fatty acid desaturation was enriched among genes downregulated following FR supplementation (Figure 6). The different branches of amino acid metabolism showed differential enrichment among up versus downregulated genes. Terms associated with branched chain amino acid (BCAA) synthesis, and serine-glycine-cysteine group synthesis were overrepresented among FR upregulated genes while aromatic amino acid synthesis was overrepresented among FR downregulated genes. Likewise, while the analysis suggests a general and long-term downregulation of secondary or specialized metabolism, and particularly of isoprenoid and phenylpropanoid metabolism, there was an enrichment of genes associated with flavonoid and, specifically, anthocyanin metabolism among genes upregulated after FR treatment. Hormone metabolism also showed a pathway-specific enrichment. Brassinosteroid and ethylene metabolism terms were overrepresented among downregulated genes while gibberellin (GA) and jasmonate (JA) metabolism terms were overrepresented among upregulated genes (Figure 6).

qRT-PCR confirms increased expression of genes associated with mobilization of starch into soluble sugars

The largest functional group of genes represented by these overrepresented parent GO terms is major CHO metabolism. This overrepresentation was also supported by a more stringent GO analysis carried out by inputting the associated AGI codes of basil genes that were identified as being differentially regulated by five-fold or more during FR treatment into the Panther functional classification tool to analyze for enrichment among the GO-Slim Biological Process annotation set (Mi et al., 2019). For upregulated genes, “monosaccharide metabolic process” was enriched 9.48-fold ($p = 0.0005$); and its parent terms, “carbohydrate metabolic process” and “metabolic process” were enriched 4.37-fold ($p = 0.00002$) and 1.49-fold ($p = 0.04$) respectively. Given the previously-demonstrated importance of soluble sugars, particularly, raffinose and stachyose, in chilling tolerance in many species (Tarkowski and

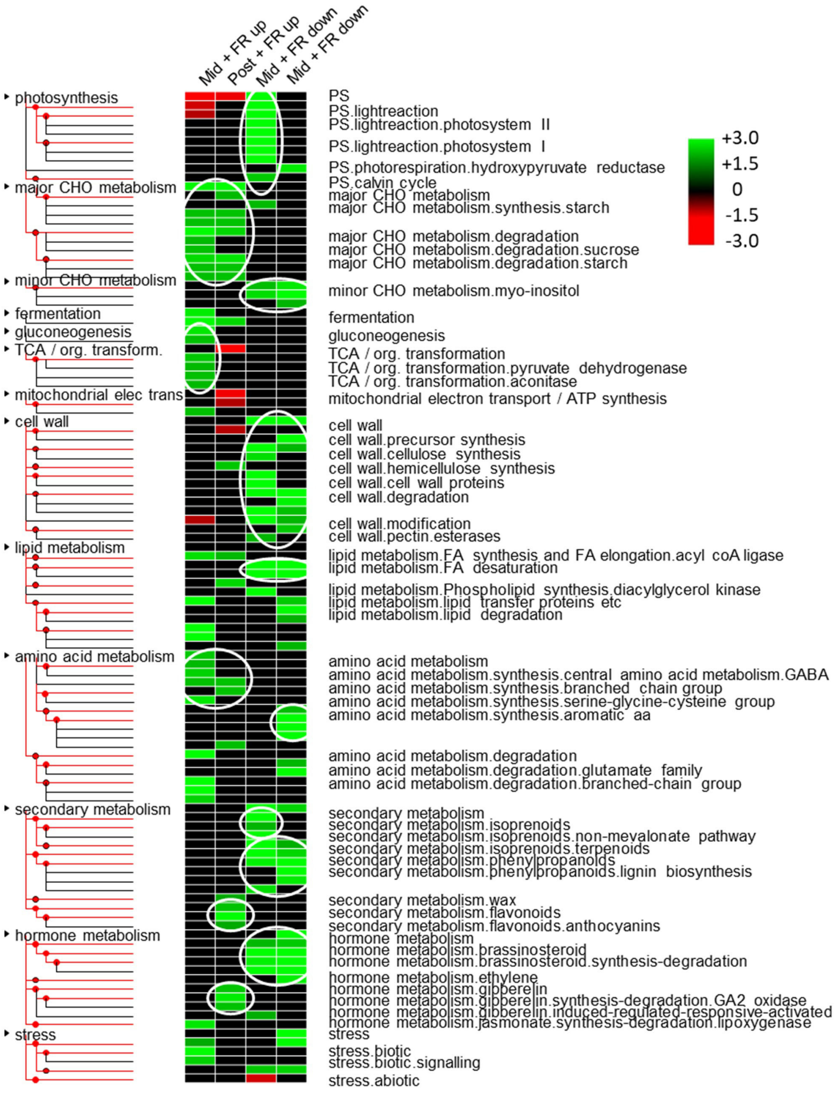


FIGURE 6

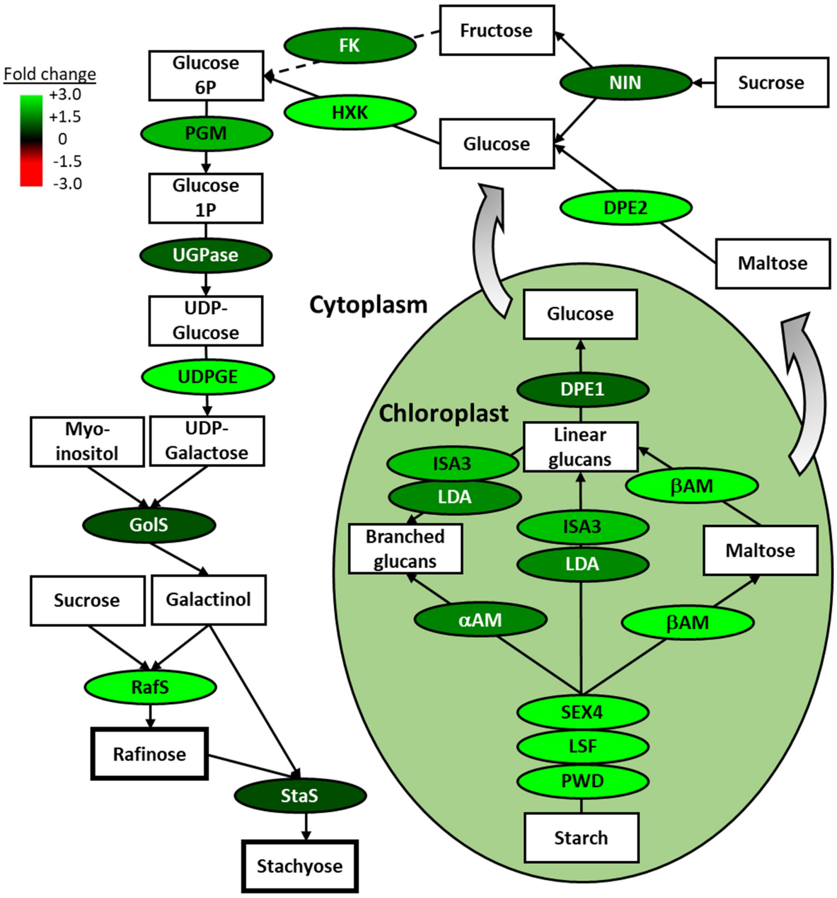
Multiple metabolic, signalling and stress-responsive pathways are affected by end-of-production midday supplementary FR. Over and under-represented MapMan gene ontology categories found among up and down-regulated genes based on pairwise comparisons between control and FR-treated plants during treatment (Mid) and 2 h after treatment (Post). Colored boxes indicate statistically-significant, over-represented categories (p-value below 0.05 based on Fisher's exact test). The color scale represents z-score, with green indicating gene ontology categories that are over-represented, and red indicating under-represented categories. Text alongside each row provides the MapMan annotation. Non-significant categories are not shown. Tree branches show "parent" and "child" gene ontology terms. White ovals indicate key gene ontology category term branches showing coordinated patterns of enrichment.

Van den Ende, 2015), we overlaid the expression patterns of genes identified within our basil transcriptome onto these metabolic pathways. Forty-five genes were identified within the major CHO metabolism functional group in our basil transcriptome, allowing the majority of enzymic steps leading from starch to raffinose and stachyose to be covered. All enzymic steps without exception showed some degree of upregulation at the level of gene expression (Figure 7; Supplementary Figure 4).

In order to confirm the patterns of gene expression observed in our RNAseq analysis, seven genes in the carbohydrate metabolism pathways represented in Figure 7 were selected for confirmatory qPCR analysis. RNA was extracted from plants taken from three independent biological experiments replicating the treatments applied in the RNAseq analysis. In all cases mean expression patterns seen via qPCR qualitatively replicated those observed in the RNAseq analysis (Figure 8) and confirmed that the changes in gene expression highlighted in RNAseq data for these genes represent significant changes. The close relationship between the RNAseq data and qPCR for these sugar metabolism genes, furthermore, validates the wider patterns of gene expression associated with the induction of cold tolerance by periodic mid-day FR treatment at end of production.

Discussion

We have demonstrated the effectiveness of four days of daily periodic 4 h mid-day supplementary FR illumination applied at end



FK	**Fructokinase**
GolS	**Galactinol Synthase**
HXK	**Hexokinase**
NIN	**Neutral Invertase**
PGM	**Phosphoglucomutase**
RafS	**Raffinose Synthase**
SPS	**Sucrose Phosphate Synthase**
StaS	**Stachyose Synthase**
UDPGE	**UDP-Glucose epimerase**
UGPase	**UDP Glucose phosphorylase**

αAM	**α-amylase**
βAM	**β-amylase**
DPE	**Disproportionating Enzyme**
ISA3	**Isomerase 3**
LSF2	**Like SEX Four 2**
LDA	**Limit Dextrinase**
SEX4	**Starch Excess 4**
GWD	**Glucan Water Dikinase**
PWD	**Phosphoglucan Water Dikinase**

FIGURE 7
Gene expression associated with production of raffinose family oligosaccharides is strongly upregulated by end-of-production midday supplementary FR treatment. Overview of starch breakdown and soluble sugar metabolic pathways leading to raffinose family oligosaccharides showing fold change in gene expression for key enzymes in basil plants during end-of-production midday supplementary FR treatment. Enzymes are indicated in ovals and metabolic products are indicated in rectangles. Fold change in expression for genes encoding the indicated enzymes as measured by RNAseq is shown relative to untreated plants along with cytoplasmic or chloroplastic location of the enzymic reaction. Scheme based on a scheme from Tarkowski and Van den Ende (2015).

of production in triggering cold tolerance in potted basil. Basil is very susceptible to chilling injury, with short duration exposure to temperatures of even 12 °C able to cause wilting and blackening of leaves (Fratianni et al., 2017). Susceptibility of basil to chilling injury creates a major challenge for the transport of plants during winter and early spring. Conveyance from glasshouse to market requires heated conditions which adds a cost to production. Warm conditions are, furthermore, not ideal for the transport of more hardy plants, meaning that the optimum conditions for these more temperate crops are then compromised. Short term storage at the point of sale prior to display can also result in exposure to cold temperatures and result in damage causing loss of appeal to the consumer and reduction in shelf life in cold-susceptible live plants.

Daily, periodic FR illumination, applied for four days at the end of production, led to a dramatic reduction in visible signs of chilling injury in potted basil following a prolonged 24 h treatment at 4 °C. At a physiological level, periodic daily FR illumination led to a striking reduction in electrolyte leakage, an indicator of membrane damage, and greatly reduced levels of stress-associated ROS accumulation in response to cold. The treatment also mitigated the impact of cold on antioxidant levels, one of the key health benefits of basil consumption.

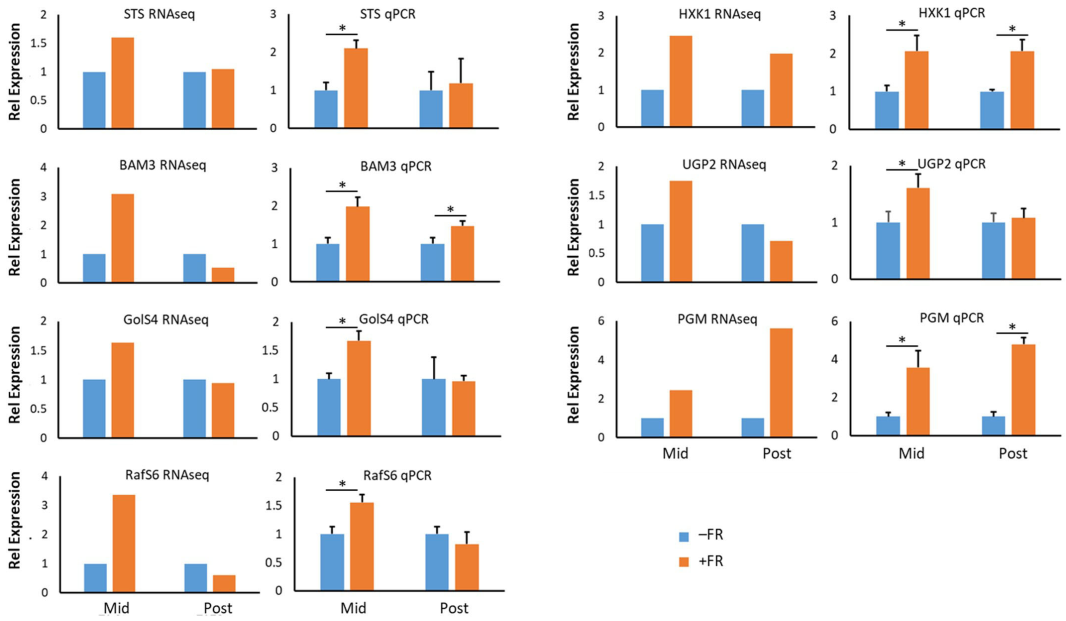


FIGURE 8
qPCR confirmation of RNAseq expression patterns in basil treated with end-of-production midday supplementary FR. Change in expression of selected genes encoding enzymes involved in starch breakdown and soluble sugar metabolic pathways leading to raffinose family oligosaccharides in basil. Expression during (Mid) and 2 h after (Post) midday supplementary FR is shown relative to untreated plants. RNAseq data is shown alongside qPCR data for each gene. qPCR data are mean ± SE for a minimum of 3 independent biological replicates. Gene symbols used are those of Arabidopsis orthologues. BAM3 – β-amylase 3, RafS6 –Raffinose synthase 6, GolS4 – Galactinol synthase 4, HXK1 – Hexokinase, PGM – Phosphoglucomutase, STS – Stachyose synthase, UGP2 – UDP-glucose pyrophosphorylase. Asterisks represent significant differences, $p \leq 0.05$.

Chilling injury causes similar physiological changes in numerous other horticultural crops. For example, cellular damage leading to electrolyte leakage has been observed in the fruit of tomato, cucumber and eggplant (Tatsumi et al., 1981; King and Ludford, 1983; Gao et al., 2015); antioxidant loss in tomato and cucumber (Hariyadi and Parkin, 1991; Imahori et al., 2016) and accumulation of reactive oxygen species in tomato, cucumber and eggplant (Lukatkin, 2002; Zaro et al., 2014; Imahori et al., 2016). Similarly, the overt changes, wilting and discoloration of leaves, are common in ornamental plants such as poinsettia (Staby et al., 1978). The end-of-production, periodic, midday FR illumination examined here could constitute a feasible approach to induce cold tolerance in other greenhouse-grown horticultural crops such as these. Certainly, constant FR supplementation in a controlled environment growth facility has been shown to induce chilling resilience in tomato as it did in fresh cut basil (Wang et al., 2016; Larsen et al., 2023). However, the periodic midday, end-of-production, FR illumination shown to be effective here for potted basil has the advantage that it would be viable in large-scale greenhouse cultivation. Only the proportion of the crop that was at the end of production stage would need to be illuminated at any one time and only short four-hour duration treatments would be required for just four days, minimizing capital and energy costs. Produce showing chilling injury is routinely discarded for not meeting consumer expectations, resulting in considerable wastage. The majority of the 50 highest-traded fruit and vegetable commodities globally are susceptible to chilling injury which has been estimated to result in annual wastage valued at over USD 100 million (Albornoz et al., 2022). Similarly, heated transport and storage cause additional costs for both growers and distributors meaning that the approach documented here could have wide significance for the horticulture industry.

Comparative transcriptomic analyses of basil plants during and after FR treatment was used to monitor potential long term physiological changes induced by FR treatment. We identified 9,728 transcripts that showed high homology to proteins expressed in Arabidopsis, allowing us to perform a pathway level analysis of the response. In terms of pattern of response, the largest group of genes was those showing a transient response while the plants were subject to the supplementary FR light. Including both up and downregulated genes, 13.4% of the expressed genome showed a rapid transient response to shade treatment, while approximately a quarter of those genes showed prolonged regulation beyond the cessation of the supplementary FR. Immediately upon removal of FR illumination, the R:FR would return to ambient levels leading to an immediate restoration of the phytochrome photoequilibrium in favour of the Pfr form. mRNA levels of key phytochrome-responsive genes involved in shade avoidance have been shown to rapidly revert to basal levels within one hour following the removal of supplementary FR (Roig-Villanova et al., 2006), consistent with an immediate reversal of shade effects on elongation growth (Cole et al., 2011). However, clearly, a significant impact of the FR supplementation persists beyond that consistent with the observed ongoing effect observed for chilling tolerance.

The application of mid-day supplementary FR illumination was previously shown to activate the *CBF* transcription factor pathway in Arabidopsis, conferring freezing tolerance (Franklin and Whitelam, 2007). The additional involvement of the CBF pathway in chilling tolerance in a number of plant species (Thomashow, 2010) suggested that this might also provide a mechanism to improve chilling tolerance in basil. Indeed, key circadian clock components, including CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), are known to regulate cold-responsive CBF pathway gene expression by acting as transcriptional activators only during the day (Kidokoro et al., 2009). In tomato, the prolonged growth of plants in monochromatic FR or in mixed R and FR light to generate a low R:FR ratio throughout the photoperiod, which has been shown to induce chilling tolerance, was demonstrated to involve FR-responsive *CBF* gene induction, also involving JA and ABA signaling (Wang et al., 2016). However, we were unable to detect any Arabidopsis CBF gene homologues in our transcriptome where the p-value of the top match was below $1x10^{-4}$ and the bit score above 50 (Pearson, 2013). This contrasts with the findings of Zhan et al. (2016) who identified a number of CBF response pathway genes in the related American basil, *O. americanum*, via a wider homology search. The majority of these had a low identity match to the species searched so it is possible that our pathway level analysis approach which, though necessity, used the Arabidopsis proteome as a reference, did not pick up *O. basilicum* homologues because of the greater evolutionary distance to Arabidopsis. However, the study by Zhan et al. (2016), which was aimed at characterizing the *O. americanum* response to cold, detected no change in expression of the key *COR* gene targets of the CBF pathway, consistent with basil's poor acclimation response to cold. Our assay was carried out in a 12 h photoperiod during winter when chilling injury during transport is most likely, but we predict that the approach will also be suitable to confer chilling tolerance in longer photoperiods. The 4 h treatment in the middle of the day was given between 4 h and 8 h after dawn which was the period shown by Franklin and Whitelam (2007) to be time of maximal FR-responsiveness of the cold response pathway in Arabidopsis. Underlying that is the work of Fowler et al. (2005) and Harmer et al. (2000) who showed that, in Arabidopsis, the key cold-regulating *CBF3* gene showed a peak of circadian expression at this time in a 12 h photoperiod. We checked publicly available Arabidopsis diurnal gene expression datasets available via the "Diurnal" web tool (Mockler et al., 2007) and confirmed that the *CBF3* gene continue to cycle in both long (16 h) and short (8 h) photoperiods (Supplementary Figure 5), therefore, supporting the proposal that periodic daytime FR supplementation will also be effective in these photoperiods. Crucially, however, the peak of *CBF3* gene expression in Arabidopsis remained consistent at 8 h after dawn in these datasets irrespective of the photoperiod suggesting that this same period between 4 h and 8 h after dawn may also be the key time for application of supplemental FR in order to induce cold tolerance in longer or shorter photoperiods. Our assay used FR supplementation which achieved an R:FR of 0.16, consistent with that used by Franklin and Whitelam (2007). It would also be interesting to determine whether a less extreme low R:FR ratio could also confer cold tolerance in basil. Any reduction in R:FR ratio below 0.8 has been demonstrated to be effective in inducing shade avoidance (Smith, 1982). Consistent with this, Wang et al. (2016) demonstrated that chilling tolerance in tomato could also be triggered by growth in light with a R:FR of 0.5. It will be possible to investigate in the future whether less intense periodic midday supplementary FR illumination would also be effective in inducing chilling tolerance in basil as this would make the treatment even more cost-effective for a commercial setting.

Pathway analysis based on our transcriptomic data showed that FR supplementation triggered a downregulation of photosynthesis-related gene expression during illumination. Acclimation in photosynthetic processes is classically associated with the shade avoidance response (Casal, 2012). However, 2h after the cessation of FR supplementation, we no longer observed any enrichment of photosynthetic processes among downregulated genes, suggesting that there would not be any long-term detriment of the 4 h FR supplementation treatment in terms of photosynthate accumulation. Our assay of ROS levels, however, may reflect an additional effect of supplementary FR on the photosynthetic machinery. We observed a small increase in H_2O_2 in leaves in FR-treated plants. Some of the additional wavelengths in the 700 – 800 nm range would be absorbed by the photosystems (Pettai et al., 2005) and it may be that the higher level of light absorption caused the formation of some additional ROS due to photo-oxidative stress. A transient increase in TCA cycle-related gene expression during supplementary FR illumination was also observed in our pathway analysis. This also concurs with previous metabolite analysis in Arabidopsis which revealed higher levels of TCA intermediates in response to shade (Yang et al., 2016; Krahmer et al., 2021). However, again, this alteration was not observed to persist in our assay 2 h beyond the removal of the FR illumination.

Interestingly most other pathways showing enrichment in our analysis showed persistent changes in gene expression. The most dramatic enrichment was seen among upregulated genes associated with major carbohydrate metabolism. The shade avoidance response is known to cause significant changes in metabolism and recent metabolic analyses have also shown that phytochrome modulates the balance between starch and sugar metabolism in Arabidopsis Krahmer et al., 2021). This is particularly significant given the key involvement of alteration of sugar metabolism in the cold acclimation response (Tarkowski and Van den Ende, 2015). Indeed, this is known to be one of the key downstream targets of CBF signaling in Arabidopsis (Tarkowski and Van den Ende, 2015). Notably, despite not seeing any alteration in expression of CBF pathway components in American basil in response to cold, Zhan et al. (2016) did find an over-representation of genes associated with starch and sucrose metabolism among genes differentially regulated in response to cold in *O. americanum* and these were proposed to be involved in chilling tolerance. Similarly, Larsen et al. (2023) in their assay applying supplementary FR to basil throughout the photoperiod for one or three weeks prior to harvest in a vertical farm set-up, demonstrated observed increased levels of soluble sugars and proposed that this could be central to the acquisition of chilling tolerance.

Monosaccharides, generally, improve osmotic protection but raffinose family oligosaccharides (RFOs) have particularly been implicated in providing tolerance to chilling, potentially protecting membrane stability, particularly of the chloroplast membranes (Nägele and Heyer, 2013; Tarkowski and Van den Ende, 2015) and also scavenging reactive oxygen species (Couée et al., 2006). Phytochrome has previously been shown to influence synthesis of rafinose (Yang et al., 2016; Krahmer et al., 2021) and, in our assay, all transcripts coding for enzymes involved in the pathway from starch utilization through to synthesis of the key RFOs, the trisaccharide raffinose, and the tetrasaccharide stachyose, were upregulated in in response to FR supplementation (Figure 7; Supplementary Figure 4). This included expression of the gene encoding galactinol synthase, the first committed and rate limiting step in RFO production. Although the upregulation of expression was not persistent beyond FR treatment for all of the specific RFO enzymes (unlike sugar metabolism more generally), it is expected that resultant enzyme levels and effects on RFO sugars would persist for some time beyond the FR treatment. Overexpression of galactinol synthase has been demonstrated to confer increased stress resistance in Arabidopsis and in chickpea (Taji et al., 2002; Salvi et al., 2018) and, more specifically, chilling tolerance in rice (Shimosaka and Ozawa, 2015), while metabolites of the raffinose pathway were also shown to accumulate as part of cold acclimation in strawberry (Koehler et al., 2015). A persistent downregulation of transcripts associated with cell wall metabolism was also observed in response to supplementary FR. Some links between cell wall metabolism and cold tolerance have recently been suggested (Xu et al., 2020) but little is known in terms of any mechanistic links. However, phytochrome is known to balance the allocation of resources between growth and resilience (Devlin, 2016) meaning this could represent a simple reallocation of resources, particularly carbohydrates, from cell wall production towards RFO production.

Alterations in fatty acids have also commonly been associated with cold acclimation, with desaturation of membrane fatty acids reducing the tight packing of membrane lipids and counteracting the decreased membrane fluidity associated with cold temperatures (Ruelland et al., 2009). Our transcriptomic analysis revealed persistent downregulation of fatty acid metabolism as a whole but particularly, we observed an enrichment of the ontology term, fatty acid desaturation, among genes downregulated following FR supplementation. Similarly, alterations in levels amino acids are also associated with improved cold tolerance. For example, proline, in particular, is known to act as an osmoprotectant to help stabilize proteins (Ruelland et al., 2009). Although our GO term enrichment analysis did not identify any enrichment of genes involved in proline biosynthesis among differentially-regulated genes, terms associated with BCAA synthesis, and serine-glycine-cysteine group synthesis were overrepresented among FR upregulated genes while aromatic amino acid synthesis was overrepresented among FR downregulated genes. Mass spectrometric analysis of cold acclimation in strawberry (Koehler et al., 2015) also found that the BCAA branch of amino acid biosynthesis, leading from pyruvate to the structures of isoleucine, leucine, and valine, was upregulated. The authors suggest that, while BCAAs are not generally associated with cold response, they are possibly also of general osmotic protective value in the same way as proline. Accumulation of serine has also previously been observed as part of a cold acclimation response in *Lolium perenne* (Bocian et al., 2015).

The observed downregulation of aromatic amino acid metabolism may be associated with a general downregulation of specialized metabolism. Shikimate pathway derived amino acids tyrosine and phenylalanine are the building blocks of phenylpropanoid biosynthetic pathways. Genes encoding phenylpropanoid and biosynthetic enzymes are strongly enriched among persistently downregulated genes. Such pathways are important in synthesis of key basil phenylpropanoid volatiles such as methyl chavicol and eugenol and the valuable polyphenol antioxidant, rosmarinic acid. Again, this concurs with the aforementioned mass spectrometric analysis of cold acclimation in strawberry which showed phenylpropanoid levels decreased in roots in response to cold in strawberry (Koehler et al., 2015). Likewise, isoprenoid metabolism also appears to be downregulated. Despite the downregulation of phenylpropanoid metabolism, there is an enrichment of genes associated with flavonoid and anthocyanin metabolism among genes upregulated after FR treatment. Anthocyanins are generally associated with cold tolerance in plants (Janská et al., 2010) and a mass spectrometric analysis in Arabidopsis identified an upregulation in anthocyanins as a key part of cold acclimation (Schulz et al., 2016). There are also both short term and persistent effects of supplementary FR on isoprenoid metabolism. The non-mevalonate (non-MVA) or methyl-D-erythritol phosphate (MEP) pathway, comprising early steps in the isoprenoid pathway is enriched among short term downregulated genes but not among persistently FR-downregulated genes suggesting it likely returns to normal following the cessation of the FR supplementation. This pathway contributes to the synthesis of key isoprenoid building blocks that got on to form a wide range of key plant molecules such as terpenoids, carotenoids and gibberellins. However, later steps of the terpenoid pathway, which include terpene synthases, show longer term downregulation suggesting that synthesis of key basil terpene volatiles such as linalool and 1,8-cineole may be downregulated. Again, this may represent a reallocation of resources from specialized metabolism towards resilience.

There was also a persistent effect of FR supplementation on the expression of genes in a number of hormone biosynthesis pathways. GO terms associated with brassinosteroid metabolism were overrepresented among persistently downregulated genes while GA and JA metabolism terms are overrepresented among upregulated genes. The FR-induction of chilling tolerance in tomato observed by Wang et al. (2016) was found to be dependent on ABA and JA signaling. The indicated upregulation of JA metabolism in our study suggests that there may be a parallel in basil in terms of JA involvement in FR-induced chilling tolerance. However, no enrichment of genes involved in ABA biosynthesis was observed among our differentially-regulated genes and this agrees with measurements of ABA carried out by Larsen et al. (2023) following growth of basil in supplementary FR for one or three weeks. Conversely, though, Larsen et al. (2023) also saw no change in JA levels in basil under these conditions raising the possibility that changes in actual levels of these hormone may be subtle if they are involved in FR-induced cold acclimation in basil. GAs have previously been shown to be a key part of cold acclimation in plants, with the accumulation of protective sugars having been shown to be downstream of reduced GA levels (Tarkowski and Van den Ende, 2015). This downregulation of GAs in cold acclimation is mediated via the upregulation of specific GA-deactivating GA-2-oxidase (*GA2OX*) genes (Colebrook et al., 2014). Indeed, homologues of gibberellin 2-oxidase 2 (*GA2OX2*) and gibberellin 2-oxidase 1 (*GA2OX1*) were both strongly upregulated in a persistent manner in our assay (Supplementary Table 2).

The GO term, brassinosteroid metabolism, was enriched among persistently downregulated genes following FR. Brassinosteroids are derived from sterols (Andrzej et al., 2020) and a closer inspection of the specific downregulated genes tagged in this pathway revealed that this enrichment actually reflects a downregulation of sterol metabolism, with the homologues of squalene epoxidase 1 (*SQE1*), *SQE2* and *SQE3*all showing substantial downregulation (Supplementary Table 2). Homologues of cycloeucalenol cycloisomerase (*CPI1*) and cycloartenol synthase 1 (*CAS1*), the enzymes catalyzing the later, first committed steps of brassinosteroid synthesis (Du et al., 2022) showed little or no response to supplementary FR. Sterols are associated with increased membrane fluidity and so, generally, decreased sterols are associated with reduced cold tolerance (Du et al., 2022). For example, a reduction in sterol content aggravated the cold stress injury of wheat (Valitova et al., 2019). However, the squalene epoxidases of the sterol biosynthetic pathway are also a key part of the pathway of triterpene synthesis (Olofsson et al., 2011) so this downregulation may be associated with reduced synthesis of triterpenes such as oleanolic acid in basil as part of the wider downregulation of specialized metabolism including essential oil and phenylpropanoid metabolism as would be expected associated with the reallocation of resources towards resilience seen in response to supplementary FR (Devlin, 2016).

Summary

Our study, building upon previous work indicating that induction of cold response pathways by FR is gated by the circadian clock (Fowler et al., 2005; Franklin and Whitelam, 2007), demonstrated that 4 h of supplementary FR given around midday in a 12 h photoperiod for only 4 days prior to end of production was able to induce cold tolerance in potted basil. The treatment was able to confer tolerance to 24 h of 4 °C temperature exposure in living plants, sufficient for transport of pots to market and short-term storage at a retailer after unloading. The method offers a non-invasive approach to induce chilling tolerance which is suitable for application in a large-scale commercial glasshouse, requiring only 4 days of end-of-production treatment and conferring cold tolerance without induction of shade avoidance responses outside of the 4 h treatment window. The short duration of treatment also makes this a relatively energy efficient treatment. The treatment significantly reduced the occurrence of visible chilling damage including wilting and discoloration while also dramatically reducing physiological markers of chilling damage such as electrolyte leakage and reactive oxygen species accumulation. Benefits were also demonstrated in terms of reducing the loss of antioxidant levels.

Transcriptomic analysis revealed persistent increases in expression of genes involved in a number of pathways widely associated with cold tolerance. Upregulation was observed for genes involved in major carbohydrate metabolism and, in particular, the synthesis of RFO sugars; genes in pathways associated with the biosynthesis of branched chain and serine-glycine-cysteine group amino acids; and genes involved in anthocyanin synthesis, all of which have previously been proposed to have protective roles in cold stress (Figure 9). Persistent downregulation was observed for genes in pathways associated with growth and, in particular, cell wall metabolism, and genes associated with specialist metabolism, in particular, phenylpropanoids and isoprenoids, potentially reflecting the commonly observed trade-off between growth and

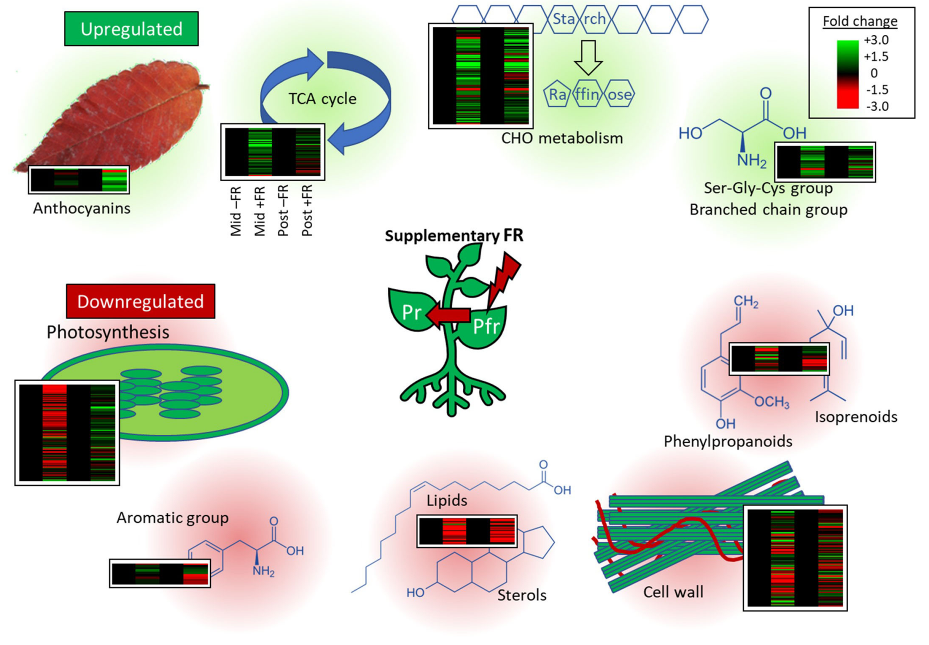


FIGURE 9
Expression of individual transcripts in key pathways differentially regulated by end-of-production midday supplementary FR. Heatmaps show RNAseq expression patterns of all transcripts within significantly enriched MapMan gene ontology categories identified among up- or downregulated genes. For each heatmap, columns represent expression in FR-treated (+FR, columns 1 and 3) relative to untreated (−FR, columns 2 and 4) plants at the same timepoint, either during (Mid, columns 1 and 2) or 2 h after (Post, columns 3 and 4) supplementary FR treatment. Each transcript is represented by a separate line. Central figure represents the effect of supplementary FR on phytochrome photoequilibrium. Gene ontology categories enriched among upregulated genes are shaded in green. Gene ontology categories enriched among downregulated genes are shaded in red.

resilience associated with acclimation to stress. A short-term downregulation of genes associated with photosynthesis and upregulation of genes associated with the TCA cycle was also observed during supplementary FR treatment but these did not persist beyond the 4 h treatment period. Given that susceptibility to chilling injury creates a major challenge for the transport of many live herbaceous plants during winter and early spring, it will be interesting to determine whether the approach has general applicability to other cold sensitive species.

Data availability statement

The data presented in the study are deposited in the NCBI SRA repository, accession number PRJNA995591.

Author contributions

FB, GS and SS contributed to experimental design and carried out the experiments. SB and AS contributed to experimental design and supervised the project. PD conceived the original idea, contributed to experimental design, supervised the project and wrote the bulk of the manuscript. All authors discussed the results and contributed to the final manuscript.

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Conflict of interest

Author SB was employed by the company Vitacress Herbs. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare that this study received funding from the company Vitacress Herbs. The funder had the following involvement in the study: SB contributed to experimental design and supervision of the project.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1239010/full#supplementary-material

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EDITED BY
Giao Nguyen,
Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia

REVIEWED BY
Filippos Bantis,
University of Patras, Greece
Fan Xxiaoxue,
Jiangsu Academy of Agricultural Sciences (JAAS), China

*CORRESPONDENCE
Rubén Moratiel
ruben.moratiel@upm.es

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Net CO_2 assimilation rate response of tomato seedlings (*Solanum lycopersicum* L.) to the interaction between light intensity, spectrum and ambient CO_2 concentration

Rubén Moratiel[1,2]*, Raúl Jimenez[2,3], Miriam Mate[4], Miguel Angel Ibánez[5], Marta M. Moreno[6] and Ana M. Tarquis[1,7]

[1]CEIGRAM, Universidad Politécnica de Madrid, Madrid, Spain, [2]AgSystems, ETSI Agronómica, Alimentaria y Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain, [3]Entomología Aplicada a la Agricultura y la Salud, Departamento de Biotecnología Microbiana y de Plantas, Centro de Investigaciones Biológicas Margarita Salas (CIB), CSIC, Madrid, Spain, [4]ICEI, Universidad Complutense de Madrid, Pozuelo de Alarcón, Madrid, Spain, [5]Departamento Economía Agraria, Estadística y Gestión de Empresas, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM), Ciudad Universitaria, Madrid, Spain, [6]University of Castilla-La Mancha, Higher Technical School of Agricultural Engineering in Ciudad Real, Ciudad Real, Spain, [7]Grupo de Sistemas Complejos, Universidad Politécnica de Madrid, Madrid, Spain

Artificial lighting is complementary and single-source lighting for controlled Environment Agriculture (CEA) to increase crop productivity. Installations to control CO_2 levels and luminaires with variable spectrum and intensity are becoming increasingly common. In order to see the net assimilation of CO_2 based on the relationship between the three factors: intensity, spectrum and CO_2 concentration, tests are proposed on tomatoes seedling with combinations of ten spectra (100B, 80B20G, 20B80G, 100G, 80G20R, 20G80R, 100R, 80R20B, 20R80B, 37R36G27B) seven light intensities (30, 90, 200, 350, 500, 700 and 1000 $\mu mol \cdot m^{-2}\ s^{-1}$) and nine CO_2 concentrations (200, 300, 400, 500, 600, 700, 800 and 900 ppm). These tomato seedlings grew under uniform conditions with no treatments applied up to the moment of measurement by a differential gas analyzer. We have developed a model to evaluate and determine under what spectrum and intensity of light photosynthesis the Net assimilation of CO_2 (A_n) is more significant in the leaves of tomato plants, considering the CO_2 concentration as an independent variable in the model. The evaluation of the model parameters for each spectrum and intensity shows that the intensity has a more decisive influence on the maximum A_n rate than the spectra. For intensities lower than 350 $\mu mol \cdot m^{-2}\ s^{-1}$, it is observed that the spectrum has a greater influence on the variable A_n. The spectra with the best behaviour were 80R20B and 80B20R, which maintained A_n values between 2 and 4 ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) above the spectra with the worst behaviour (100G, 80G20R, 20G80R and 37B36G27R) in practically all situations. Photosynthetic Light-Use Efficiency (PLUE) was also higher for the 80B20R and 20R80B spectra with values of 36,07 and 33,84 mmol $CO_2 \cdot$mol photon^{-1}, respectively, for light intensities of 200 µmol·m^{-2} s^{-1} and 400 ppm of CO_2that increased to values of 49,65 and 48,38 mmol CO_2·mol photon^{-1} for the same light intensity and concentrations of 850 ppm. The choice of spectrum is essential, as indicated by the data from this study, to optimize the photosynthesis of the plant species grown in the plant factory where light intensities are adjusted for greater profitability.

KEYWORDS

photosynthesis, light intensity, light spectrum, CO_2 concentration, net CO_2 assimilation rate, tomato seedling

1 Introduction

Light is one of the major factors that drive photosynthesis and plant development. Light spectra, intensity and duration (light dimensions) are involved in almost all vegetative processes. Among others, photomorphogenesis, phototropism, maintenance of the circadian clock or the Shade-Avoidance Syndrome (SAS) (Trojak et al., 2022). These light dimensions are also directly responsible for the efficiency of photosynthesis and determine the Net CO_2 Assimilation Rate (A_n). This balance fixes plants' photo-assimilate amount and phytochemical content (Spalholz et al., 2020). Since the beginning of the century, scientific publications regarding Light Emitting Diode (LED) illumination in plants have grown exponentially, given the fine-tuning of light that new technology provides (Sipos et al., 2020). This increase manifests the amount of research performed lately, testing the effect of different dimensions of light over many crops (Viršilė et al., 2017; Sipos et al., 2020), which has been proven to be not only species- but even cultivar-dependant, each reacting differently (even though with some general similarities) to the spectra, intensity and photoperiod they were exposed (Bantis et al., 2018; Liang et al., 2021).

Artificial illumination has become relevant in the last decades as supplemental and sole-source illumination for Controlled Environment Agriculture (CEA) to increase crop productivity (Bantis et al., 2018). The recent LED technology development allows not only the reduction of costs and, therefore, the increase of the efficiency of vegetable production but also the establishment of the effect of narrow wavelength spectra over different plant processes, as mentioned above. Moreover, with LED technology, it is possible to change the most important aspects of light that affect plants: photosynthetic photon flux density (PPFD) in the photosynthetically active radiation (PAR) spectral, photoperiod, lighting mode (impulses or continuous) and light spectral composition (Berkovich et al., 2017).

In general, Red (R; 600-700 nm) and Blue (B; 400-500 nm) wavebands (RB) are the most efficient in terms of photosynthesis. They comprehended the *in vitro* absorption peaks of Chlorophyll *a* (430 nm and 662 nm) and Chlorophyll *b* (453 nm and 642 nm) when they were extracted in diethyl ether (Du et al., 1998; Pennisi et al., 2019). That is why different RB light combinations were first used as LED growing illumination (Spalholz et al., 2020; Zheng et al., 2021). Green (G; 500-600 nm) and some wavebands outside the Photosynthetically Active Radiation (PAR; 400-700 nm) range, such as Far Red (FR; 700-800 nm), have only recently started to be taken into consideration for these artificial illumination solutions since they appear to be poorly absorbed by photosynthetic pigments (Zhen et al., 2021). These authors consider that a new definition should replace the definition of PAR (400-700 nm) extended PAR (ePAR,400-750nm), which is more influential in photosynthesis and plant growth and development (Zhen et al., 2021). However, these wavebands are of importance in photosynthesis at conditions of high PPFD due to their higher transmittance within the leaves and canopy or by balancing excitation of Photosystem II (PSII) and Photosystem I (PSI) in the so-called Emmerson effect (Zhen et al., 2019). The effect of these wavelengths over plant development has shifted the light composition of artificial illumination solutions, which are starting to include broad-spectrum LEDs to cover all PAR wavebands and somehow mimic sunlight (Berkovich et al., 2017).

Being able to control the intensity and spectrum that plants receive is crucial in order to harness photosynthetic processes. It is now known that light quantity and quality have an interactive effect on photosynthesis driven by the transmittance and absorption properties of different wavelengths within the PAR spectrum (Terashima et al., 2009). Given the high absorptance of RB by the chlorophylls *in vitro*, it has been commonly accepted that they are the main drivers of photosynthesis, especially when compared to G light (van Iersel, 2017). However, this only seems true under low PPFD conditions when the photosynthetic machinery is not saturated. The low transmittance of RB light does not allow those photons to penetrate deeper leaf layers. So they are absorbed by chlorophylls even when they are already saturated, forcing them to

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Abbreviations: A_n, Net CO_2 Assimilation rate; B, Blue photons (400 – 500 nm); G, Green photons (500-600 nm); LED, light-emitting diode; PLUE, Photosynthetic Light-Use Efficiency; PPFD, photosynthetic photon flux density; PAR, photosynthetically active radiation; R, Red photons (600-700 nm).

dissipate that energy non-photochemically on the adaxial layers of the leaf. On the other hand, chlorophylls' low absorptance of G light allows it to reach chloroplast through the whole leaf. Thus increasing the photosynthetic light use efficiency once PPFD is high enough to start saturating the upper layers of leaves (Terashima et al., 2009).

In order to dissect the interactive effect of light quality and intensity, a comprehensive study was presented quantifying the photosynthetic response of lettuce to different combinations of B, G and/or R light over a wide range of intensities (Liu and van Iersel, 2021). It was demonstrated that G photons could drive photosynthesis as efficiently as B light under low PPFD conditions. However, given their low absorptance, G light is generally less efficient in these conditions. However, at high PPFD, the photosynthetic efficiency of G light was similar to R light, not only once absorbed but on a light incident basis, with B light scoring the lowest. Similar behaviour in sunflowers on the effect of the green spectrum was reported by Terashima et al. (2009). Chlorophylls, flavonoids, and carotenoids absorb blue light, which may lead to a lesser photosynthetic yield once chlorophylls are saturated (Sun et al., 1998). This phenomenon occurs to G light on a lower basis, which might explain why R light continues to have the best behaviour. As PPFD increases, the yield for CO_2 assimilation per photon decreases as more energy is dissipated in non-photochemical processes. However, this reduction seems slower under G light than under B or R light, assumably because of the lower absorption of green photons, thus, their better distribution throughout the leaf. This more uniform distribution reduces non-photochemical quenching (NPQ). At the same time, lower penetration of blue and red light upregulates NPQ on the upper parts of the leaves and cannot drive photosynthesis on the lower levels (Liu and van Iersel, 2021). This is important under high PPFD since NPQ is proportional to light intensity (Zhen and van Iersel, 2017).

Tomato (*Solanum lycopersicum* L.) is one of the crops most cultivated worldwide (FAOSTAT, 2022) due to its nutritional characteristics and culinary importance (Dorais et al., 2008). It is also a model plant for the study of the effect of light on plants in controlled environments, given its responsiveness to light (Yang et al., 2018). Light availability in greenhouse crops along seasons is a growing concern in northern latitudes and meridional areas such as the Mediterranean. It has been proven that supplemental LED inter-lighting illumination (R:B, 3:1) results in larger and heavier tomato fruits, especially in seasons with lower solar radiation, as well as faster fruit growth and maturation, which in turn results in higher yields (Paucek et al., 2020). This might be due to the photosynthetic capacity and light sensibility of unripped tomato fruits, which have been shown to increase their melatonin levels under RB light, a novel plant hormone that seems to promote ripening by inducing ethylene production and protect against senescence by scavenging reactive oxygen species (Li et al., 2021)

The main climate factors determining plant growth are ePAR light (Zhen et al., 2021), air temperature, air humidity, CO_2 concentration, wind, root temperature, nutrient availability, water and oxygen. The chemical reaction of photosynthesis can be simplified as follows (Equation 1):

$$light\ \ energy + \ 6CO_2 + 12H_2O = C_6H_{12}O_6 + 6O_2 + 6H_2O \qquad (1)$$

Carbon dioxide is one of the substrates for photosynthesis. Thus, it can be a limiting factor for the reaction when its concentration is below optimal. According to the Law of Minimum (also known as Liebig Law), varying only the light energy plants receive may not be enough to enhance photosynthesis properly since it is not the only substrate of the reaction. Thus, it is necessary to consider ambient CO_2 to evaluate the photosynthetic efficiency of a given light source, adding a new dimension to the light quality and intensity interactive effect. In protected crop conditions, the environmental factors modified last are CO_2 and lighting, the temperature and relative humidity being the first to be controlled.

In this study, we aim to identify how light intensity, its spectrum and concentration of ambient conditions of CO_2 affect the Net CO_2 Assimilation in tomato (*Solanum lycopersicum* L.) plants. Tomato seedlings grew under uniform conditions with no treatments applied up to the moment of measurement. Tomato leaves were exposed to spectra of different combinations of blue, green and/or red light in a wide range of intensities and increasing CO_2 availability to assess the Net CO_2 Assimilation under each ambient condition.

2 Materials and methods

2.1 Plant material

The trials were conducted at the Experimental Field at Agricultural Engineering School of Universidad Politécnica de Madrid (Latitude: 40.439413N; Longitude: 3.737547W) during May-Dic 2021. Tomato (*Solanum lycopersicum* L. cv. *Anairis*) seeds were sown in trays of 36 pots (3 cm length x 3 cm wide x 7 cm depth) filled with seedbed substrate with a mixture composed of 70% of white peat and 30% black peat (Tray 70/30 Gramoflor GmbH & Co. KG, Vechta, Germany) and covered with vermiculite. All plants were cultivated in a glass Greenhouse at the Experimental Field with an ACOM 2019® (Acom, Balsicas, Murcia, Spain) environmental controller. The mean night/day temperature fluctuated between 18-14°C/28-20°C with adifference in day and night temperature (DIF) between +6 and +10°C and humidity between 80-60%. The maximum light intensity in the greenhouses was 400 $\mu molm^{-2}s^{-1}$ (shade screens and application of calcium hydroxide, whitening, on the cover material were used) and day-night photoperiod of 14-10 h. Pots were watered daily as needed, and once a week, a general nutritive solution (5.69 mM $CaNO_3$; 2.77 mM KNO_3; 4.08 mM $MgSO_4$; 1.56 mM K_2PO_4 and 0.048 gL^{-1} Nutrel C micronutrients Yara Inc.), was used to avoid nutrient deprivation. The conductivity of the nutrition solution was 2.1 $dS{\cdot}m^{-1}$ and a pH of 6.2. Seedlings were grown to BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) 14-15, 4-5th leaf on the main shoot unfolded (Feller et al., 1995). One day before taking the measurements, the seedlings were moved to a climatic chamber with a capacity of 350 L (Mod. Hot-Cold GL, JP Selecta, Barcelona, Spain). The conditions in the chamber were 25 °C, 80% relative humidity, and PPFD of 400 μmol/m^2·s with the photoperiod 14-10 h day-night.

2.2 Carbon assimilation measurements

Tomato plants were taken for measurements 25-35 days after sowing. Only plants whose at least a fourth true leaf was completely unfolded and whose third true leaf did not show any sign of stress or deprivation were selected for analysis and discarded afterwards. Selected plants were dark-adapted for 30 minutes, and their third leaf was clipped to the leaf cuvette (PLC 3 Universal Leaf cuvette) with a window measuring 25 mm x 7 mm of a gas exchange system (CIRAS-3, PP Systems, Amesbury, MA, USA) provided with a LED Light Unit (RGBW). This dimmable light unit peaks at 446 nm (blue), 523 nm (green) and 653 nm (red) with full width at half maximum (FWHM) of 16, 36 and 17 nm, respectively (Liu and van Iersel, 2021). The combination of blue, green and red light allowed for the composition of 10 different light spectra (Table 1). The three monochromatic spectra of PAR radiation (100B, 100G, 100R), six combinations of binary spectra based on percentages of blue 20%, that are used in supplemental lighting (Kaiser et al., 2019) maintaining the proportions of 20%/80% of all combinations of blue, green and red spectrum and simulated natural light (reference of our study). Three plants were measured per spectrum. Each light spectrum was tested at seven different light intensities (30, 90, 200, 350, 500, 700 and 1000 μmol·m^{-2} s^{-1}).

Different spectra were designed so it would be possible to determine the effect of each monochromatic light as well as their interaction by pairs. A trichromatic spectrum was designed to average the light a plant would receive on a sunny summer day. Therefore, solar radiation was recorded in triplicate at three different moments of a sunny summer day (morning, noon and evening) using a spectroradiometer (PN-200, UPRtek, Zhunan Township, Miaoli County, Taiwan) and those nine readings were averaged. The resulting spectrum was then divided into segments of 100 nm, and the fraction Blue (400-499 nm), Green (500-599 nm) and Red (600-699 nm) was calculated and used to design the trichromatic spectrum (Figure 1).

TABLE 1 Light composition of each spectrum used in the study.

Spectra	Fraction of photon flux (%)		
	Blue	Green	Red
100B	100	0	0
80B20G	80	20	0
20B80G	20	80	0
100G	0	100	0
80G20R	0	80	20
20G80R	0	20	80
100R	0	0	100
80R20B	20	0	80
20R80B	80	0	20
37R36G27B	37	36	27

To study the photosynthesis efficiency under different spectra, intensities, and CO$_2$ concentrations, we constructed CO$_2$ response curves for each intensity and spectrum using a Rapid A/Ci Response (RACiR) technique (Saathoff and Welles, 2021). The photosynthetic light-use efficiency (PLUE) was calculated, which is defined as the slope between the net CO$_2$ assimilation rate (A$_n$) and incident PPFD on the leaf.

After 5 minutes of acclimatization in the lowest CO$_2$ concentration and light intensity (200 ppm CO$_2$, 30 μmol·m^{-2}·s^{-1} photons), three Net CO$_2$ Assimilation rates (A$_n$), Stomatic Conductance, Vapour Pressure Deficit (VPD) and Water Use Efficiency (WUE) readings were taken at a 10 seconds interval. CO$_2$ concentration was then raised to 100 ppm, and the leaf was kept in these conditions for two minutes before recording the three readings. This continued through all the CO$_2$ concentrations studied (200, 300, 400, 500, 600, 700, 800 and 900 ppm). Once the maximum concentration is reached, the light intensity rises to the next lowest intensity of the study. CO$_2$ concentration then decreases by 100 ppm per triplicate of readings until the lowest concentration is reached, and then light intensity rises again. This process is repeated until all light intensities (30, 90, 200,350, 500, 700 and 1000 μmol·m^{-2}·s^{-1}) are reached. Recordings are taken for every CO$_2$ concentration and light intensity in the study (Table 2). Environmental conditions inside the cuvette were controlled by the leaf gas exchange system setting values of leaf temperature of 25.0 ± 0.4°C and VPD of 1.6 ± 0.3kPa.

2.3 Statistical analysis

A nonlinear mixed effects model (Lindstrom and Bates, 1990) was estimated to relate assimilation rate as a response variable and light spectra, light intensity and CO$_2$ concentration levels as explanatory variables.

An asymptotic regression model was used to describe limited growth, where the response variable approaches a horizontal asymptote as CO$_2$ approaches infinity.

The model used was:

$$A_n = c + (d - c) \times (1 - e^{-\frac{CO_2}{b}}) \tag{2}$$

Where

A_n is the Net CO$_2$ Assimilation rate, c is the value of A$_n$ when the CO$_2$ level is zero, d is the maximum attainable A$_n$, 1/b is proportional to A$_n$'s relative rate of increase as CO$_2$ increases, and e is a random error term. This term (e) was assumed to have a normal distribution with zero mean and different variance for each intensity level.

It is assumed that the values of c, d and e depend on the light spectra and intensity levels.

c = Intensity + Spectra
d = Intensity + Spectra + u
b = Intensity + Spectra

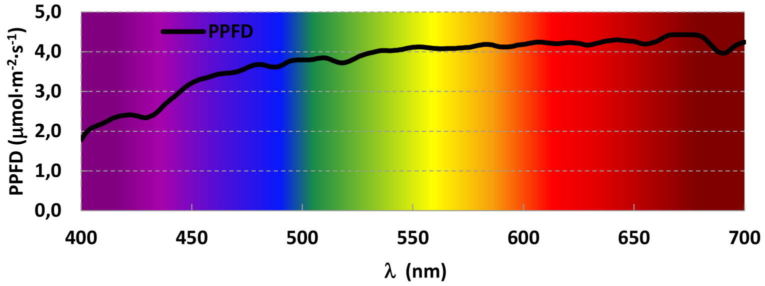

FIGURE 1
Averaged solar radiation in the interval of photosynthetic active radiation (PAR) during a sunny summer day (August 6, 2021) in the Experimental Field in Madrid, Spain (Latitude: 40.439413 N, Longitude: 3.737547W).

where u is a random term that considers the variability for each plant in the parameter d. The random term u was assumed to have a normal distribution with mean 0 and variance σ_u^2.

The statistical model, as defined, took into account the hierarchical structure in which the data was obtained: Three plants per spectrum were measured, and each plant was tested at different light intensities and CO_2 concentrations. The experimental data estimated the parameters b, c and d based on the intensity and spectrum levels used. Hypothesis tests were performed to determine significant differences between their estimates and standard errors. Normality assumptions were also checked using the residuals of the estimated model. The bigger the b parameter, the lower the curvature; hence, the higher the theoretical CO_2 saturation point. The more intensity applied, the higher the d parameter and the highest theoretical maximum A_n is reached. This model studies the effect of the different spectra and intensities over the c, d and b parameters.

Statistical analysis was performed in the R environment (R Core Team, 2021). The model estimation was done with the *nlme* package (Pinheiro et al., 2021), a testing hypothesis was carried out with the *emmeans* package (Russell, 2022) and graphics with the ggplot package (Wickham, 2009).

TABLE 2 Environmental conditions (CO_2 Concentration and Light Intensity) were set for each set of three readings (N) during measurements.

N	[CO_2] (ppm)	I (µmol·m^{-2}·s^{-1})	N	[CO_2] (ppm)	I (µmol·m^{-2}·s^{-1})	N	[CO_2] (ppm)	I (µmol·m^{-2}·s^{-1})
1	200	30	20	500	200	39	800	500
2	300	30	21	600	200	40	900	500
3	400	30	22	700	200	41	900	700
4	500	30	23	800	200	42	800	700
5	600	30	24	900	200	43	700	700
6	700	30	25	900	350	44	600	700
7	800	30	26	800	350	45	500	700
8	900	30	27	700	350	46	400	700
9	900	90	28	600	350	47	300	700
10	800	90	29	500	350	48	200	700
11	700	90	30	400	350	49	200	1000
12	600	90	31	300	350	50	300	1000
13	500	90	32	200	350	51	400	1000
14	400	90	33	200	500	52	500	1000
15	300	90	34	300	500	53	600	1000
16	200	90	35	400	500	54	700	1000
17	200	200	36	500	500	55	800	1000
18	300	200	37	600	500	56	900	1000
19	400	200	38	700	500			

This has been performed over three plants per spectrum described in Table 1.

3 Results

3.1 Changes in net carbon assimilation due to varying CO_2 concentration, light intensity and spectra used

Net Carbon Assimilation (A_n) was assessed at eight different CO_2 concentrations for seven light intensity values at ten light spectra varying R, G and B light fractions (Figure 2) on the third true leaf of tomato plants. For every spectrum, at light intensities of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or higher, A_n/CO_2-concentration response showed the typical display of an asymptotic curve, A_n rising rapidly as CO_2 increased at lower levels until reaching a concentration in which A_n increase slows down and even stops going up. The higher the intensity, the higher the curvature, reaching higher A_n values in all spectra. At lower light intensities (30 and 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$), CO_2 response curves were more lineal, not showing a pronounced change in the tendency of the curve. The curves' shapes were similar at all the spectra and intensities used, pointing out the same A_n behaviour due to increases in CO_2 concentration. However, the absolute values of A_n changed through different spectra. The highest A_n values at every light intensity were observed at 20R80B and 80R20B spectrums. The lowest A_n values were archived by the 20G80R spectrum, followed by the trichromatic spectrum 37R36G27B (Figure 2). The highest A_n values, 18.9 μmol $CO_2 \cdot m^{-2} \cdot s^{1}$, were obtained at CO_2 concentrations of 700, 800 and 900 ppm, and with 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity and in 80R20B spectrum. Contrary, the A_n lowest values, -4.9 and -3.2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, were reached in 100 G and 20G80R spectrums, and CO_2 intensities of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 200 ppm, respectively.

3.2 Model

The most frequently used methods to understand how C_3 plant photosynthesis responds to changes in CO_2 concentration are based on the studies of Farquhar et al. (1980). These biochemical models focus on the activity of ribulose 1:5 bisphosphate carboxylase/ oxygenase (Rubisco). We have developed a model to determine under which light photosynthesis spectrum and intensity is greater for tomato plants' leaves, considering the concentration of CO_2 as an independent variable.

Table 3 studies the interference of the model with the intensity of illumination. The simulated solar spectrum of 37R36G27B is a reference for the analysis. The intensity of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is used as a reference to analyze the spectra (Table 4). The same trend is observed in each spectrum or intensity compared. It shows an increase or decrease of the parameters by the same amount (Table 5).

In the analyses carried out in the model, one of the most important parameters is to determine *d* (asymptotic value of maximum A_n when the CO_2 concentration tends to infinity), with a higher value of *d*, higher production potential. Table 3 shows the estimated values of *d* for each lighting intensity level. It is observed that there is a positive relationship between the intensity and the values of d. The increase in intensity tends to increase the estimated value of the parameter *d*. The highest intensities, 700 and 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, show the highest values of parameter d (12.01 and 11.98, respectively), showing significant differences for the other intensities. This trend would be observed regardless of the spectrum used, decreasing or increasing the estimated values by the same amount depending on the spectrum used. The estimated values

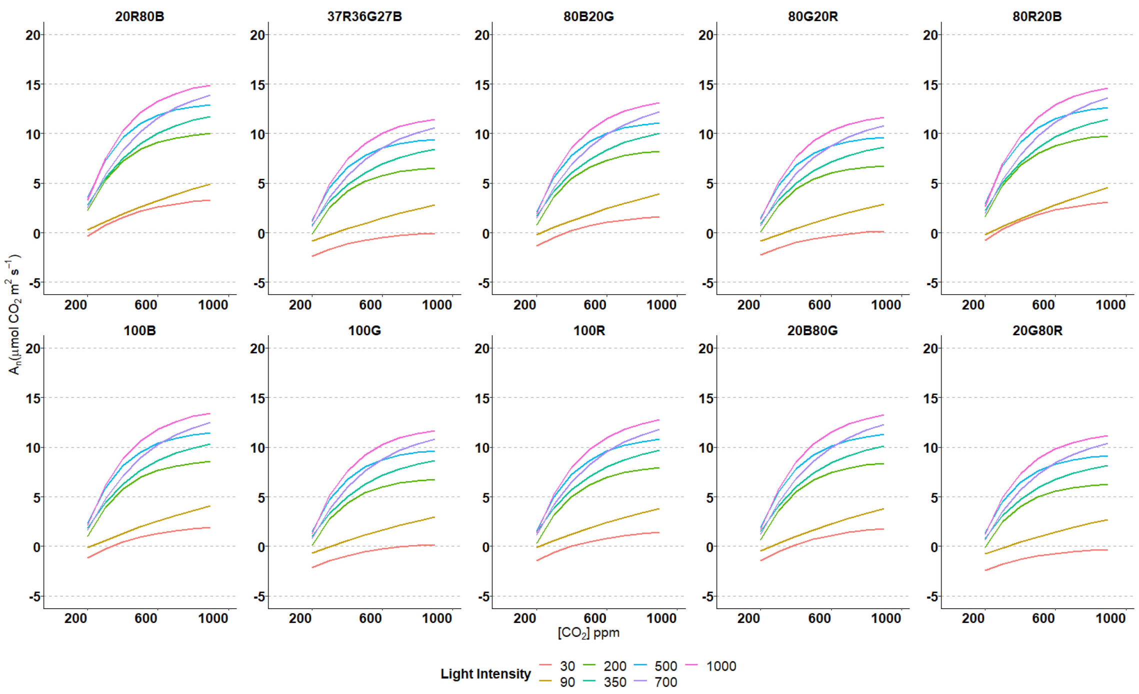

FIGURE 2
Estimated nonlinear regression models for A_n and CO_2 concentrations at different intensities and spectrums used.

TABLE 3 Mean parameters *d* and *b* values from the model (Equation 2) for every intensity examined in the spectrum 37R36G27B.

Intensity (μmol·m^{-2}·s^{-1})	*d* (± s.e.)		*b* (± s.e.)	
30	0.26^{a}	± 0.42	320.71^{abc}	± 43.90
200	6.74^{bcd}	± 0.39	202.98^{abc}	± 8.13
90	7.73^{bcd}	± 1.50	1264.96^{d}	± 249.9
350	9.28^{c}	± 0.42	310.32^{c}	± 16.08
500	9.68^{c}	± 0.39	202.34^{a}	± 7.83
1000	11.98^{d}	± 0.4	231.78^{b}	± 8.61
700	12.01^{d}	± 0.4	333.32^{c}	± 17.34

Mean values ± standard error. Mean values that include a common letter in the same column are not statistically different ($p \leq 0.05$).

of parameter *b* (responsible for curvature) fluctuate between 202.34 for 500 µmol·m^{-2}·s^{-1} and 1264.96 for 90 µmol·m^{-2}·s^{-1} (Table 3). Note that all the intensities, except for 90 µmol·m^{-2}·s^{-1}, are between 200 and 340. For intensity of 90 µmol·m^{-2}·s^{-1}, very high *b* values are observed, indicating that it practically approaches its maximum linearly. At higher values of *b*, the curve tends to be more linear and needs higher levels of CO_2 to reach its maximum asymptotic value. It is observed that the *b* values do not follow an intensity pattern. However, at low intensities (30 and 90 µmol·m^{-2}·s^{-1}), this parameter shows more significant fluctuations, as the standard error values point out, being much higher than those of the higher intensities (Table 3).

Table 4 shows the model's behaviour depending on the light spectrum for an intensity of 350 µmol·m^{-2}·s^{-1}. As a function of the spectrum, the d and b parameters range values are 8.96 to 12.82 and 305.77 to 334.68, respectively. These values are significantly lower than those required by the light intensity (*d* from 0.26 to 12.01 and *b* from 202.34 to 1264.96). It is observed how the spectra 80R20B and 20R80B are the ones that would reach the highest potential values of A_n, with significant differences concerning the other spectra. The spectrum that reaches the lowest maximum A_n are 20G80R, 37R36G27B, 80G20R, 100G and 100R, with no significant differences (Table 4). Parameter *b* is a parameter with few fluctuations due to the spectra, with no significant differences between 20G80R, 37R36G27B, 80G20R, 100G 80B20G, 20B80G, 100B, 80R20B and 20R80B. In addition, another group is formed by 80B20G, 20B80G, 100B, 80R20B, 20R80B and 100R without significant differences.

Table 5 shows the model parameter values (*d*, *b and c)* for each light intensity and spectrum used in this experiment. Trichromatic spectrum 37R36G27B at 350 µmol·m^{-2}·s^{-1} has been chosen as a reference since it was designed as sunlight radiation. Its values have been used as the baseline. The curve can be obtained for each intensity and spectrum in Table 5.

Figure 3 compares the models with two PPFDs and two spectra. It is observed how the -PPFD component influences more than the spectra. However, the spectra show different trends with the same intensity, observing differences in A_n among them.

When applying values from Table 5 to Equation 2. values for A_n can be calculated for each intensity and spectrum for any fixed CO_2 concentration (Table 6). This work is particularised for three possible scenarios of CO_2 concentration taken into consideration based on different real-life scenarios that can occur under a greenhouse (Both et al., 2017). The first scenario is the study of the A_n of the spectra for the atmospheric concentration (400 ppm), and the second case is the increase in carbon fertilization up to

TABLE 4 Mean ± values of parameters *d* and *b* from the model (Equation 2) for every spectrum light intensity of 350 µmol·m^{-2}·s^{-1}.

Spectra	*d* (± s.e.)		*b* (± s.e.)	
80R20G	8.96^{a}	± 0.42	305.77^{a}	± 16.07
37R36G27B	9.28^{a}	± 0.42	310.32^{a}	± 16.08
20R80G	9.49^{ab}	± 0.42	308.37^{a}	± 16.04
100G	9.51^{ab}	± 0.42	310.90^{a}	± 16.03
100R	10.85^{ab}	± 0.42	334.68^{b}	± 16.21
20G80B	11.06^{cd}	± 0.42	321.73^{ab}	± 16.06
80G20B	11.27^{cd}	± 0.42	325.59^{ab}	± 16.02
100B	11.41^{cd}	± 0.42	320.69^{ab}	± 16.02
80R20B	12.63^{de}	± 0.43	321.12^{ab}	± 15.91
20R80B	12.82^{e}	± 0.42	314.45^{ab}	± 15.88

Mean values ± standard error (s.e.). Mean values that include a common letter in the same column are not statistically different ($p \leq 0.05$).

TABLE 5 Values of parameters *d*, *b* and *c* from the model of Equation 2 for every spectrum and intensity tested.

Spectra	Parameter	Intensities (µmol·m^{-2}·s^{-1})							
		30	90	200	350	500	700	1000	△
37R36G27B	***d***	0.26	7.73	6.74	9.28	9.68	12.01	11.98	-
	b	320.71	1264.96	202.98	310.32	202.34	333.32	231.78	-
	c	-4.62	-2.34	-11.61	-6.89	-12.88	-8.76	-13.74	-
100B	***d***	2.39	9.86	8.87	11.41	11.81	14.14	14.11	2.13
	b	331.08	1275.33	213.35	320.69	212.71	343.69	242.15	10.37
	c	-4.12	-1.84	-11.11	-6.39	-12.39	-8.26	-13.24	0.50
100G	***d***	0.49	7.96	6.97	9.51	9.91	12.24	12.21	0.23
	b	321.3	1265.55	203.57	310.91	202.93	333.91	232.37	0.59
	c	-4.45	-2.17	-11.45	-6.73	-12.72	-8.60	-13.57	0.17
100R	***d***	1.83	9.3	8.31	10.85	11.25	13.58	13.55	1.57
	b	345.07	1289.32	227.34	334.68	226.7	357.68	256.14	24.36
	c	-3.97	-1.69	-10.96	-6.24	-12.23	-8.11	-13.09	0.65
20B80G	***d***	2.25	9.72	8.73	11.27	11.67	14.00	13.97	1.99
	b	335.98	1280.23	218.25	325.59	217.61	348.59	247.05	15.27
	c	-4.50	-2.22	-11.49	-6.77	-12.76	-8.64	-13.62	0.12
20G80R	***d***	-0.06	7.41	6.42	8.96	9.36	11.69	11.66	-0.32
	b	316.17	1260.42	198.44	305.78	197.8	328.78	227.24	-4.54
	c	-4.47	-2.19	-11.46	-6.74	-12.73	-8.61	-13.59	0.15
20R80B	***d***	3.8	11.27	10.28	12.82	13.22	15.55	15.52	3.54
	b	324.85	1269.1	207.12	314.46	206.48	337.46	235.92	4.14
	c	-3.88	-1.60	-10.87	-6.15	-12.14	-8.02	-13.00	0.74
80B20G	***d***	2.04	9.51	8.52	11.06	11.46	13.79	13.76	1.78
	b	332.13	1276.38	214.4	321.74	213.76	344.74	243.2	11.42
	c	-4.11	-3.60	-3.09	-2.58	-2.07	-1.56	-1.05	0.51
80G20R	***d***	0.47	7.94	6.95	9.49	9.89	12.22	12.19	0.21
	b	318.76	1263.01	201.03	308.37	200.39	331.37	229.83	-1.95
		-4.63	-4.64	-4.65	-4.66	-4.67	-4.68	-4.69	-0.01
80R20B	***d***	3.6	11.07	10.08	12.62	13.02	15.35	15.32	3.34
	b	331.52	1275.77	213.79	321.13	213.15	344.13	242.59	10.81
	c	-4.44	-2.16	-11.43	-6.71	-12.70	-8.58	-13.56	0.18

Δ show the change rate of that parameter with baseline 37R36G27B spectrum and 350 µmol·m^{-2}·s^{-1}.

levels of 850 ppm, a situation that can be frequently reached in the carbon fertilization of greenhouses of crops of C_3 metabolism like rose and tomato. The last scenario is the reduction of the CO_2 concentration to levels of 200 ppm, a situation that can occur at certain times of the day with poor ventilation in greenhouses and a high rate of photosynthesis in crops with high LAI (Leaf Area Index).

Blue-containing spectra show higher A_n values than their Red and Green counterparts, followed by red-containing spectra. G light seems to have a lower effect in enhancing Net Carbon Assimilation. The highest values for A_n are archived by the 20R80B spectrum, followed by the 80R20B spectrum and then by the monochromatic 100B compared to other spectra at the same light intensity and CO_2 concentrations. The lowest A_n values belong to the 20G80R spectrum, followed by the trichromatic 37R36G27B. Table 6 shows that under conditions of low CO_2 concentration (200 ppm), the A_n values begin to be positive at incident PPFD of 200 µmol·m^{-2}·s^{-1}, although spectra such as 20R80B take positive

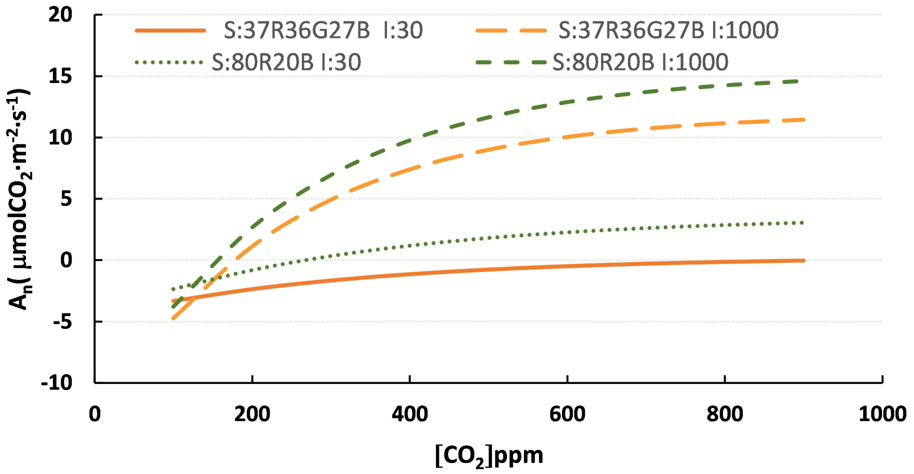

FIGURE 3
Curves of the spectra model 80R20B and R37G36B27 and PPFD of 30 and 1000 µmol·m^{-2}·s^{-1}. S:R37G36B27 I:30, spectra R37G36B27 at 30 µmol·m^{-2}·s^{-1}; S:R37G36B27 I:1000, spectra R37G36B27 at 1000 µmol·m^{-2}·s^{-1}; S: 80R20B I:30, spectra 80R20B at 30 µmol·m^{-2}·s^{-1} and S:80R20B I:1000, spectra 80R20B at 1000 µmol·m^{-2}·s^{-1}.

values at 90 µmol·m^{-2}·s^{-1}. The A_n values do not exceed 4 µmolCO$_2$·m^{-2}·s^{-1} at these CO$_2$ concentrations and any PPFD. The highest values are reached in the 80R20B and the 20R80B spectra (2.66 and 3.31, respectively).

For values of 400 ppm of CO$_2$, even at intensities of 30 µmol·m^{-2}·s^{-1}, positive A_n values are observed for all spectra except for 37R36G27B, 100G and 20G80R. For concentrations of 400 ppm of CO$_2$ with PPFD of 350 µmol·m^{-2}·s^{-1}, the spectra that reached 7 µmol CO$_2$·m^{-2}·s^{-1} were 80R20B and 20R80B. The same trend is obtained for these two spectra at concentrations of 850 ppm of CO$_2$ and 350 µmol·m^{-2}·s^{-1} of PPFD, where they are the only ones that reach 11 µmolCO$_2$·m^{-2}·s^{-1} of A_n.

In Table 7, a relative comparison is made taking as reference the A_n of 350 µmol·m^{-2}·s^{-1}, with 400 ppm of CO$_2$ and spectrum of 37R36G27B (with a value of 4.82 µmolCO$_2$·m^{-2}·s^{-1}) and determined the percentages related to this situation Equation 3. The values shown result from the value obtained as a reference minus the value divided by the reference and multiplied by 100. In this case, it can be seen how the values of the 20R80B and 80R20B spectra are always higher than the reference and other spectra, although it will depend on the PPFD and the CO$_2$ concentration. The 20R80B and 80R20B spectra with a lower light intensity of 150 µmol·m^{-2}·s^{-1} than the reference (reference with 350 µmol·m^{-2}·s^{-1} and type of spectra with 200 µmol·m^{-2}·s^{-1}) show values of A_n that are 50 and 40% higher, respectively.

$$A_{n\ relative} = 100 * \left(\frac{A_{n\ i} - A_{n\ reference}}{A_{n\ reference}} \right) \qquad [3]$$

Where

$A_{n\ reference}$ = Value of A_n with spectrum 37R36G27B with a PPFD of 350 µmol·m^{-2}·s^{-1} and CO$_2$ concentration of 400 ppm. A_{ni} = Value of A_n with spectra, PPFD and CO$_2$ concentrations selected according to Table 6.

Although the relative increases in A_n are marked mainly by the intensity of light and the concentration of CO$_2$. Table 7 shows the spectra's influence on the Net Carbon Assimilation. Values in A_n with PPFD conditions of 1000 µmol·m^{-2}·s^{-1} and 400 ppm of CO$_2$ in the 37R36G27B spectrum are similar to those obtained by the 20R80B and 80R20B spectra at PPFD of 350 µmol·m^{-2}·s^{-1} with 400 ppm of CO$_2$.

Table 8 shows how the variable PLUE changes depending on the spectrum, intensity, and concentration of CO$_2$. It is observed that PLUE increases as the concentration of CO$_2$ increases analyzed. At low concentrations of CO$_2$ (200 ppm), the highest values of PLUE occur at intensities of 350 µmol·m^{-2}·s^{-1}, while as the concentration of CO$_2$ increases, the highest efficiency is reached at values of 90 -200 µmol·m^{-2}·s^{-1}. Concerning the spectra, although all of them follow the same behaviour, there are differences between them. The ones that show the best efficiency are the spectrum of 20R80B and 20B80R. Concerning light intensity, maximum PLUE values are shown for all spectra and with 200 ppm CO$_2$ in values around 200-350 PPFD, as we increase CO$_2$ to 400 and 850 ppm, the maximum PLUE values drop to 200 and 200-90 PPFD, respectively Table 6. Calculated values (according to Table 5 and Equation 2) of A_n (µmol CO$_2$·m^{-2}·s^{-1}) for each light intensity and spectrum tested at three CO$_2$ concentration scenarios: 400 ppm as atmospheric CO$_2$ concentration, 850 ppm as carbon- fertilized greenhouse concentration, and 200 ppm as the case of a CO$_2$-deprived ambient due to a high photosynthetic rate.

4 Discussion

Since McCree's work (McCree, 1971), Red and Blue light have been considered the most efficient wavebands for photosynthesis. This correlates with chlorophyll absorption spectra, which peak at about 430 and 660 nm (Viršilė et al., 2017). In the literature, no references have been found that deal jointly with the combination of the three factors of light intensity, spectrum and CO$_2$ concentrations of the photosynthetic responses of seedlings grown

TABLE 6 Calculated values (according to Table 5 and Equation 2) of A_n (µmol CO_2·m^{-2}·s^{-1}) for each light intensity and spectrum tested at three CO_2 concentration scenarios: 400 ppm as atmospheric CO_2 concentration, 850 ppm as carbon- fertilized greenhouse concentration, and 200 ppm as the case of a CO_2-deprived ambient due to a high photosynthetic rate.

Spectra	Intensity light (incident PPFD, µmol·m^{-2}·s^{-1})																				
	30	90	200	350	500	700	1000	30	90	200	350	500	700	1000	30	90	200	350	500	700	1000
	200 ppm CO_2							400 ppm CO_2							850 ppm CO_2						
37R36G27B	-2,36	-0,87	-0,11	0,79	1,28	0,61	1,13	-1,14	0,39	4,18	4,82	6,56	5,75	7,40	-0,08	2,59	6,46	8,23	9,34	10,39	11,32
100B	-1,17	-0,14	1,05	1,87	2,36	1,62	2,14	0,45	1,31	5,81	6,30	8,12	7,14	8,87	1,89	3,85	8,50	10,15	11,37	12,25	13,29
100G	-2,16	-0,69	0,07	0,97	1,46	0,79	1,31	-0,93	0,58	4,39	5,02	6,76	5,95	7,60	0,14	2,78	6,69	8,45	9,57	10,61	11,55
100R	-1,42	-0,11	0,32	1,44	1,53	1,18	1,35	0,01	1,24	4,99	5,67	7,23	6,49	7,96	1,34	3,62	7,85	9,50	10,70	11,57	12,59
20B80G	-1,47	-0,49	0,64	1,51	1,93	1,24	1,69	0,20	0,98	5,50	5,99	7,78	6,81	8,50	1,71	3,57	8,32	9,94	11,18	12,02	13,08
20G80R	-2,40	-0,77	-0,10	0,80	1,32	0,64	1,19	-1,30	0,43	4,04	4,72	6,44	5,68	7,32	-0,36	2,52	6,17	7,99	9,06	10,16	11,06
20R80B	-0,35	0,29	2,23	2,78	3,59	2,52	3,31	1,56	1,89	7,21	7,50	9,57	8,35	10,29	3,24	4,69	9,93	11,55	12,81	13,65	14,74
80B20G	-1,33	-0,18	0,80	1,69	2,11	1,45	1,91	0,20	1,23	5,48	6,03	7,79	6,88	8,55	1,56	3,69	8,15	9,82	11,01	11,92	12,94
80G20R	-2,25	-0,84	0,08	0,92	1,49	0,74	1,32	-0,98	0,44	4,41	5,01	6,79	5,94	7,64	0,12	2,69	6,68	8,45	9,56	10,61	11,55
80R20B	-0,80	-0,23	1,64	2,25	2,96	1,97	2,66	1,19	1,41	6,77	7,06	9,08	7,87	9,77	2,98	4,28	9,68	11,25	12,54	13,33	14,45

under the same conditions until measurements with spectrum change. Other authors studied plants grown in different conditions from the beginning of their growth. Authors such as Huber et al. (2021) studied the relationship between light intensities (three daily light integral, DLIs) and three different CO_2 concentrations but with a fixed spectrum ratio of 40B:60R. Other authors focus on the relationship between light intensities and light quality in spectra of red and blue combinations (Hernández and Kubota, 2012; Zheng et al., 2021).

In our tests, we have observed (Tables 3, 4) that the influence of intensity on parameter *d* (asymptotic value of maximum A_n when the CO_2 concentration tends to infinity) is higher than the effect of the tested spectra. The net assimilation rate (A_n) obtained in the trial was around between 11-15 µmol CO_2·m^{-2}·s^{-1} for 1000 µmol·m^{-2}·s^{-1} of PPFD. These values agree with those obtained by Yang et al. (2018) for tomato seedlings at 6-leaf stage. In our model, the *d* parameter values, when the intensities of 30 and 700 µmol·m^{-2}·s^{-1}, is 11.75 while the fluctuation of *d* as a spectrum function is 3.86. The effects of light intensity or PPFD is the primary variable to identify in the light needs of plants (DLI). Usually, increases in light intensity correlate with increases in net photosynthesis rate (A_n) (Bowes et al., 1972; Fan et al., 2013). PPFD of 700 µmol·m^{-2}·s^{-1} was the highest A_n obtained by Ke et al. (2022) compared to the intensity of 300 and 500 µmol·m^{-2}·s^{-1}. In our results, values of 700 and 1000 µmol·m^{-2}·s^{-1} were the highest A_n obtained, too.

However, at similar intensity levels, the effect of the spectrum greatly influences A_n (Table 5 and Figure 3). The best results were shown by the combination of red and blue LEDs (20B80R and 80R20R). Similar results were reported on tomato seedlings by (Hernández et al., 2016) after studying various spectra, concluding that the combinations of 30B70R and 50B50R showed a greater fresh and dry mass. However, there were no differences in A_n between the different spectra. Liu et al. (2011) indicated that the spectrum with the best performance in improving photosynthesis for tomato seedlings was the combination of RB in a 1:1 ratio with PPFD of 320 µmol$m^2$$s^{-1}$. Kaiser et al. (2019) indicated that in greenhouse tomato production, the optimal proportions of blue light are between 6-12%, while the higher values are the optimal plant growth. Liu et al. (2011) showed that of the monochromatic lights tested (blue, green, yellow and red), the one that showed the best behaviour was a blue light, coinciding with the results shown in this study (Table 7). Our results indicate that Blue light is more efficient in driving photosynthesis when comparing the three monochromatic light sources (100B>100R>100G). At the same time, photosynthesis is more efficient when Blue light is in combination with other colours, being the predominant wavelength of the mix. The absorbance values for Blue and Red light are between 80 and 95% (Terashima et al., 2009). Moreover, the limitation in one of these spectra causes photosynthesis inefficiency or other plant disorders (Hogewoning et al., 2010). This study has shown that monochromatic Red light impairs the photosynthetic machinery, reducing photosynthetic capacity in the so-called "red light syndrome" (Kaiser et al., 2019). This effect can be reverted by adding even small proportions of Blue light (Hogewoning et al., 2010). The peaks at which the LEDs used in this work emit light are closer to the absorption peak of chlorophylls

TABLE 7 Calculated A_n increment relative to that of the spectrum 37R36G27B (designed after sun radiation) at 350 µmol·m^{-2}·s^{-1} and 400 ppm of CO_2 (yellow cell) for every spectrum and intensity tested in the three theoretical CO_2 concentration scenarios of 200 ppm, 400 ppm and 700 ppm.

	Intensity light (incident PPFD, µmol·m^{-2}·s^{-1})																				
	30	90	200	350	500	700	1000	30	90	200	350	500	700	1000	30	90	200	350	500	700	1000
Spectra	200 ppm CO_2							400 ppm CO_2							850 ppm CO_2						
37R36G27B	-149	-118	-102	-84	-73	-87	-77	-124	-92	-13	0	36	19	53	-102	-46	34	71	94	115	135
100B	-124	-103	-78	-61	-51	-66	-56	-91	-73	20	31	68	48	84	-61	-20	76	110	136	154	176
100G	-145	-114	-98	-80	-70	-84	-73	-119	-88	-9	4	40	23	58	-97	-42	39	75	98	120	139
100R	-129	-102	-93	-70	-68	-76	-72	-100	-74	3	18	50	35	65	-72	-25	63	97	122	140	161
20B80G	-131	-110	-87	-69	-60	-74	-65	-96	-80	14	24	61	41	76	-65	-26	72	106	132	149	171
20G80R	-150	-116	-102	-83	-73	-87	-75	-127	-91	-16	-2	33	18	52	-107	-48	28	66	88	111	129
20R80B	-107	-94	-54	-42	-26	-48	-31	-68	-61	50	56	98	73	113	-33	-3	106	139	165	183	206
80B20G	-128	-104	-83	-65	-56	-70	-61	-96	-75	14	25	62	43	77	-68	-24	69	104	128	147	168
80G20R	-147	-117	-98	-81	-69	-85	-73	-120	-91	-9	4	41	23	58	-98	-44	38	75	98	120	139
80R20B	-117	-105	-66	-53	-39	-59	-45	-75	-71	40	46	88	63	102	-38	-11	101	133	160	176	200

🟥 A_n <-99   🟧 -99< A_n <-66   ▨ -66< A_n <-33   ☐ -33< A_n <33   🟩 33< A_n <66   🟩 66> A_n <99   🟩 99< A_n

The font number shows whether the calculated A_n is higher (in green) or lower (in red) than the reference. The background darkness indicates whether the An value is higher (in green) or lower (in red) than that of 37R36G27B at 350 µmol·m^{-2}·s^{-1} at the same CO_2 concentration.

TABLE 8 Photosynthetic Light-Use Efficiency (PLUE, mmol CO_2/mol photon) for each light intensity and spectrum tested at three CO_2 concentration scenarios: 400 ppm as atmospheric CO2 concentration, 850 ppm as the concentration of a carbon fertilized greenhouse and 200 ppm as the case of a CO_2 deprived ambient due to a high photosynthetic rate.

Spectra	Intensity light (incident PPFD, $\mu mol \cdot m^{-2} \cdot s^{-1}$)																				
	200 ppm CO_2							400 ppm CO_2							850 ppm CO_2						
	30	90	200	350	500	700	1000	30	90	200	350	500	700	1000	30	90	200	350	500	700	1000
37R36G27B	-78,52	-9,64	-0,55	2,26	2,57	0,87	1,13	-38,07	4,33	20,91	13,78	13,11	8,22	7,40	-2,82	28,75	32,31	23,53	18,68	14,84	11,32
100B	-38,94	-1,58	5,23	5,34	4,72	2,32	2,14	14,84	14,55	29,03	17,99	16,24	10,21	8,87	63,01	42,80	42,49	29,01	22,73	17,50	13,29
100G	-72,03	-7,66	0,37	2,79	2,93	1,13	1,31	-31,08	6,39	21,94	14,35	13,52	8,50	7,60	4,65	30,94	33,43	24,16	19,13	15,15	11,55
100R	-47,29	-1,23	1,58	4,12	3,06	1,69	1,35	0,34	13,79	24,96	16,21	14,45	9,27	7,96	44,54	40,17	39,26	27,15	21,39	16,52	12,59
20B80G	-49,07	-5,48	3,21	4,31	3,85	1,78	1,69	6,59	10,93	27,48	17,11	15,57	9,73	8,50	57,08	39,70	41,59	28,41	22,36	17,18	13,08
20G80R	-80,09	-8,59	-0,51	2,28	2,65	0,92	1,19	-43,48	4,75	20,20	13,47	12,87	8,11	7,32	-11,99	28,05	30,87	22,82	18,12	14,51	11,06
20R80B	-11,64	3,17	11,14	7,94	7,19	3,60	3,31	51,94	20,96	36,07	21,44	19,13	11,92	10,29	107,97	52,09	49,65	33,00	25,61	19,50	14,74
80B20G	-44,26	-1,96	4,02	4,84	4,22	2,07	1,91	6,52	13,65	27,42	17,23	15,58	9,83	8,55	52,14	40,99	40,74	28,05	22,03	17,03	12,94
80G20R	-75,11	-9,37	0,42	2,63	2,98	1,06	1,32	-32,80	4,93	22,05	14,32	13,59	8,49	7,64	3,85	29,89	33,40	24,14	19,12	15,15	11,55
80R20B	-26,60	-2,58	8,20	6,43	5,91	2,81	2,66	25,12	15,65	33,84	20,16	18,16	11,24	9,77	99,36	47,55	48,38	32,14	25,09	19,04	14,45
Mean	-52,36	-4,49	3,31	4,29	4,01	1,82	1,80	-4,01	10,99	26,39	16,61	15,22	9,55	8,39	41,78	38,09	39,21	27,24	21,43	16,64	12,66

in blue than in red, thus more effectively used by these pigments. This fact could explain the results obtained.

Greenlight has been proposed to drive photosynthesis more efficiently than Blue and Red light when light intensity reaches a saturating point (Terashima et al., 2009) due to the better distribution/penetration along the leaves. This effect is effectively used along the depths of the leaf and not only on the adaxial parts. However, this was not the case in this study. Greenlight reaches lower A_n values than Red and Blue light. Although, it is observed that at low intensities, the differences of A_n between Green and other spectra are more significant as the intensity of light increases (Table 6). This result could be because light saturating points have not been reached in this experiment, so all light received by leaves did not saturate the chloroplasts present on the adaxial part of leaves.

Further research should be performed at higher light intensities to determine whether higher intensities are needed to boost Green photosynthetic efficiency in tomatoes or whether this phenomenon is species-dependent and does not occur in tomato plants. One of the most critical variables in artificial lighting is PLUE, which represents the ratio between net photosynthesis and moles of photons applied. Concerning our test, it is observed that as the intensity increases, the PLUE

decreases. The values and trend shown align with those obtained by (Ke et al., 2022) with values between 30-40 with light intensities from 300 to 500 $\mu mol \cdot m^{-2} s^{-1}$ and CO_2 concentrations of 1000 ppm.

The model established in this study does not adjust properly to the cases of lower light intensity (30 and 90 µmol·m-2·s-1), showing a discreet but lineal increase of An. This might be because the CO2 saturating point is reached at low light intensities, thus skipping the exponential part of the CO2 response curves. This would be in synchrony with the assumption of not reaching the light saturation point, evidencing a high light necessity of tomato (or at least the variety studied).

5 Conclusion

The interaction between light intensity and CO_2 concentration on tomato seedlings has shown characteristic curves A_n/Light and A_n/CO_2 for all spectra. The intensity of light and the concentration of CO_2 are the parameters that most condition the A_n rate. The generated model and its parameters allow for the estimation and discrimination of the values achieved based on intensity, spectra, and CO2 concentration. For some fixed values of CO_2 concentration and with close tested light intensities, spectra with better behaviour than others have been observed, and the differences between spectra with lower light intensities were more pronounced. The spectra with better behaviour, with a higher rate of A_n, have been 20B80R and 80B20R. The tests carried out indicate that at low lighting intensities tested<350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the effect of the spectrum is more important because these increases represent a very high percentage with respect to the maximum potential of A_n. In the artificial light application industry, where the intensities are low and can never compete with those coming from natural light, spectrum choice is essential to optimize the photosynthesis of the species, as indicated by the data on photosynthetic light use efficiency in this study. It is necessary to conduct more research to evaluate the growth and development of the complete plant since, although the spectra cited (20B80R, 80B20R) show better behaviour in A_n, they can influence the morphology and growth of the plant in different ways from a crop perspective.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RM: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing, Resources, Visualization. RJ: Data curation, Formal analysis, Investigation, Writing – original draft. MMa: Data curation, Formal analysis, Project administration, Software, Visualization, Writing – original draft. MI: Data curation, Formal analysis, Methodology, Software, Validation, Writing – original draft. MMo: Conceptualization, Methodology, Resources, Writing – original draft. AT: Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – review & editing, Writing – original draft.

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Conflict of interest

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

Publisher's note

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EDITED BY
Giao Nguyen,
Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia

REVIEWED BY
Prakash P.,
Central Tuber Crops Research Institute (ICAR), India
Juan Valiente-Banuet,
Monterrey Institute of Technology and Higher Education (ITESM), Mexico

*CORRESPONDENCE
Chelsea R. Maier
✉ chelseamaier@gmail.com

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Light blocking film in a glasshouse impacts *Capsicum annuum* L. yield differentially across planting season

Chelsea R. Maier[1]*, Sachin G. Chavan[2], Norbert Klause[1], Weiguang Liang[1], Christopher I. Cazzonelli[1,2], Oula Ghannoum[1,2], Zhong-Hua Chen[1,2,3] and David T. Tissue[1,2,4]

[1]National Vegetable Protected Cropping Centre, Western Sydney University, Penrith, NSW, Australia, [2]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia, [3]School of Science, Western Sydney University, Penrith, NSW, Australia, [4]Global Centre for Land-Based Innovation, Western Sydney University, Penrith, NSW, Australia

High energy costs are a barrier to producing high-quality produce at protected cropping facilities. A potential solution to mitigate high energy costs is film technology, which blocks heat-producing radiation; however, the alteration of the light environment by these films may impact crop yield and quality. Previous studies have assessed the impact of ULR 80 [i.e., light-blocking film (LBF)] on crop yield and photosynthetically active radiation (PAR); however, an assessment of the spectral environment over different seasons is important to understand potential crop impacts through different developmental phases. In this study, two varieties (red and orange) of *Capsicum annuum* were grown across two crop cycles: one cycle with primary crop growth in the autumn (i.e., autumn experiment [AE]) and the other with primary crop growth in the summer (i.e., summer experiment [SE]). LBF reduced PAR (roof level: 26%–30%, plant canopy level: 8%–25%) and net radiation (36%–66%). LBF also reduced total diffuse PAR (AE: 8%, SE: 15%), but the diffuse fraction of PAR increased by 7% and 9% for AE and SE, respectively, potentially resulting in differential light penetration throughout the canopy across treatments. LBF reduced near-infrared radiation (700 nm–2,500 nm), including far-red (700 nm–780 nm) at mid- and lower-canopy levels. LBF significantly altered light quantity and quality, which determined the amount of time that the crop grew under light-limited (<12 mol m^{-2} d^{-1}) versus sufficient light conditions. In AE, crops were established and grown under light-limited conditions for 57% of the growing season, whereas in SE, crops were established and grown under sufficient light conditions for 66% of the growing season. Overall, LBF significantly reduced the yield in SE for both varieties (red: 29%; orange: 16%), but not in AE. The light changes in different seasons in response to LBF suggest that planting time is crucial for maximizing fruit yield when grown under a film that reduces light quantity. LBF may be unsuitable for year-round production of capsicum, and additional development of LBF is required for the film to be beneficial for saving energy during production and sustaining good crop yields in protected cropping.

KEYWORDS

light blocking film, protected cropping, energy use, light quality, light intensity, resource sustainability, *Capsicum annuum* L., agricultural technology

1 Introduction

Researchers worldwide have attempted to reduce energy use in protected cropping (PC) food production (Ali & Albayati, 2017). In some PC facilities, such as high-tech greenhouses, wavelength-selective photovoltaics have replaced glass paneling in glasshouses to capture energy from less biologically relevant wavelengths of the light spectrum to offset energy consumption, while allowing the most important wavelengths to be utilized by the crop (Loik et al., 2017; He et al., 2021). Dye-sensitized and opaque photovoltaics have been mounted on greenhouses, which shade the crop but also produce electricity (Ntinas et al., 2019; Yano and Cossu, 2019). Recently, a radiation-reducing film (light-blocking film; LBF) developed for residential buildings to reduce the transmittance of heat-producing light has been used; hence, it might be useful for reducing the energy costs associated with crop production (Chaiyapinunt et al., 2005; Chavan et al., 2020). Although wavelength-selective photovoltaics, opaque photovoltaics, and LBF can offset or reduce energy expenditure and water and fertilizer use, they may also impact the quantity and quality of the light for crop production Chavan et al., 2020; Loik et al., 2017; Yano and Cossu, 2019; Zhao et al., 2021; Lin et al., 2022). Hence, it is necessary to understand how crops respond to these changes in light quality and quantity and how energy-saving LBF can be optimized to achieve more sustainable food production in greenhouses in the future.

Light quality and quantity affect plant development, physiology, and yield (Trouwborst et al., 2010; Bugbee, 2016; Poorter et al., 2019; Zhen and Bugbee, 2020). The spectral distribution of solar radiation can be described as a continuous range of wavelengths: ultraviolet radiation (UV: 200 nm–400 nm; about 5% of global solar radiation), photosynthetically active radiation (PAR: 400 nm–700 nm; about 45%), and near infrared radiation (NIR: 700 nm–2,500 nm; about 50%) (Abdel-Ghany et al., 2012). Each of these regions has been associated with varied effects on plant development (Kami et al., 2010; Kendrick and Kronenberg, 2012).

Many studies have investigated the light spectral impacts on crop performance and growth using monochromatic light (Azad et al., 2011; Lin et al., 2013; O'Carrigan et al., 2014), which are narrow-band spectral regions of light used to measure plant photosynthetic responses. Previous studies have considered photosynthetic activity using monochromatic LED lights that would produce specific bandwidths of light and found that photosynthetic activity drops at wavelengths >700 nm (Zhen and Bugbee, 2020). However, more recent studies have found that measurements conducted under a broader spectrum of light (400 nm–725 nm) increased CO_2 assimilation (photosynthesis) by 10%–21%, suggesting that the impact of photon flux density was underestimated in the past (Zhen and Bugbee, 2020). These broader spectra are important, but these studies do not provide information on the quantity of light at each wavelength, are not easily quantifiable in greenhouses and are relatable to plant developmental responses. Scientists and researchers have not yet agreed on the numerical parameters for light quality, and more research is needed, with particular attention paid to the continuous measurement of light quality throughout the plant life cycle (Azad et al., 2011; Casierra-Posada et al., 2014).

Variations in natural light also affect plant development (Dokoozlian and Kliewer, 1996). Daily total natural light variation as measured by the daily light integral (DLI; mol photons m^{-2} d^{-1}) from 400 nm to 700 nm is correlated with changes in plant physiology, development, and nutrient composition. For instance, a meta-analysis found that DLI was positively correlated with leaf mass per area, leaf thickness, and stomatal conductance and negatively correlated with specific stem length, total leaf nitrogen, and leaf area ratio (Poorter et al., 2019); however, light quality measurements were not considered in this meta-analysis. At similar DLI levels, the spectral quality profiles may be different in different scenarios. While spectral quality varies throughout the year with changes in the solar azimuth across solar transition periods (equinoxes and solstices) (Lean and Deland, 2012), spectral quality can also shift on consecutive days at the same time of the day if clouds are present. Cloud-immersed days could reduce the total solar radiation by up to 85%. In a study investigating light quality in the forests of the Appalachian Mountains, it was found that during cloudy days, blue light was enhanced by 5%–15% at the top of the forest canopy, while transmittance to the understory was reduced by 25%–60%. On cloud-immersed days, red light decreased by 6%–11%; however, transmission of red light to the understory increased by 25%–30%. These results, while in a forest setting, indicate that clouds impact spectral quality and quantity, as well as through-canopy transmission of specific wavebands (Reinhardt et al., 2010).

Crops behave differently, depending on the relative proportion of light reduction. Overall, a reduction in cumulative light resulted in a reduction in both fresh and dry weights. Herbs grown under colored film reduced total PAR by 34% and decreased herb dry weights by 29%–40% (Hückstädt et al., 2013). In the production of cut flowers, decreased radiation reduced the number of shoots, shoot weight, and quality of roses. It has been estimated that a 1% reduction in radiation will result in a 0.8%–1% reduction in yield, with lower radiation conditions having a relatively stronger impact during the low radiation months of winter compared to summer (Marcelis et al., 2006). For lettuce under step decreases in light intensity, Kosma et al. (2013) found that fresh weight was reduced significantly at each light intensity reduction for both the winter and spring seasons. Although there are seasonal light differences in DLI maxima, crops also behave differently depending on the photoperiod ascension or descension (Heuvelink, 1995).

The proportion of diffuse light also varies with time of day and external conditions, and impacts crop plant development and photosynthesis. Photosynthesis depends on both incident light and light penetration into the canopy mass, such that photosynthesis can be higher in lower PPFD under diffuse light conditions than under direct light conditions (Hemming et al., 2006; Markvart et al., 2010). Depending on the light conditions, hazed glass, especially with the addition of a topical film, may increase the diffuse light fraction received by the crop. On high-light days, 90% of light is intercepted within the upper 50%–60% portion of the crop canopy reducing the ability of lower leaves to contribute to photosynthesis, ultimately reducing assimilate supply and thus reducing fruit yield capacity. Interlighting with LEDs has been used to overcome the impact of shaded lower canopy regions and has been shown to increase fruit yield in capsicum (Jokinen et al., 2012). Within a glasshouse, large areas of shading occur because of the structural components of the facility and light is not well-distributed to the growing plants underneath (Gruda, 2005). Most high-tech glasshouses use high-quality hazed glass to reduce these shaded areas because the hazed glass further diffuses light upon transmission. Diffuse light is incident on more surface angles that are present within the canopy than direct light due to these multiple angle points, thereby increasing total crop photosynthesis (Hemming et al., 2008). Days with a high proportion of diffuse light can also increase the total radiation due to reflectance off clouds, allowing for higher light intensity and further light penetration throughout the canopy (Priva Help Center, 2022). LBF is a film applied to hazed glass that diffuses light incident on a crop; therefore, it is important to understand how LBF impacts the diffuse light environment in a glasshouse.

Spectral quality and quantity vary significantly over short- (minutes) and long-term (months) intervals and are the key factors affecting crop production. While there are numerous reports of light impacts on PC horticultural crops, much of the research is based in low light environments in Europe where most of the design of glasshouses takes place (Montagu, K. 2018). As such, glasshouses may not be optimized for the high-light Australian horticultural industry which experiences significantly higher radiation loads (Montagu, K. 2018). In Australia, energy consumption is the second-largest cost of PC after labor costs. Hence, it is vitally important to measure the light quantity and quality regimes in PC under Australian solar and climatic conditions. LBF reduces the heat load when applied to glasshouse roofs and sidewalls, but it alters the spectral quantity and quality of light, and has been shown to affect crop development and yield of *S. melongena* and *C. annuum* (Chavan et al., 2022; T. Lin et al., 2022). However, the impact of LBF on spectral quality has not been fully assessed across a crop's lifetime nor has it been compared across different planting seasons.

Although we did not conduct an economic analysis of LBF in this study, energy costs are the second highest associated with PC production, highlighting the need to implement energy-saving techniques, products, and infrastructure in existing PC facilities (Barbosa et al., 2015). The manufacturer of LBF, Saint-Gobain, reports that energy savings with the use of their film can be up to 30% in industrial or residential settings (Solar Gard Saint-Gobain, 2023). While these estimates do not encompass the use of LBF in PC facilities, the expected energy savings from LBF are theoretically proportional to the reduction in SW radiation; however, there may be inhibitory impacts from heat transfer through convection (Chaiyapinunt et al., 2005). Reducing the energy costs for the PC industry would reduce operational costs and greenhouse gas emissions, both benefiting communities at large (Maraveas et al., 2023). Government incentives exist in Australia for the implementation of energy-saving techniques in agriculture (Grants and funding, 2023). As agricultural film technology is still developing, these government agricultural incentives are not specifically aimed at LBF-type technologies; however, adoption of energy-saving films in PC agriculture is likely to be high, as it has short-term economic benefits (Piñeiro et al., 2020). The sustainability of these products, such as longevity and the ability to be recycled, is vital to their entry into and continued use in the PC industry, as environmental impact is a key consideration among producers when adopting new technologies (Piñeiro et al., 2020).

In our study, we investigated the impact of LBF on light spectral quality and quantity, and whether this impacts the plant growth and fruit yield of *C. annuum*? We investigated the impact of LBF on light environments, including PAR, shortwave (SW) and longwave (LW) radiation, and diffuse light during two *C. annuum* crop cycles, and the impact of altered light under LBF on crop growth and yield.

2 Materials and methods

2.1 Plant materials

Capsicum is one of the top 10 vegetables by volume and the top 15 by value within Australia. While most capsicum are grown outdoors in Queensland, capsicum is increasingly grown in high-tech greenhouses year-round in Australia's cooler southern states

(Horticulture Innovation Australia, 2019). The LBF project targeted two varieties of *C. annuum* that were grown at the National Vegetable Protected Cropping Centre (NVPCC). The project consisted of two crop cycles: (1) crop grown starting in a low light (ascending photoperiod) environment (transplant date: 5 April 2019, removal date: 5 December 2019) using varieties Gina (red) and O06614 (orange); and (2) crop grown starting in a high light (descending photoperiod) environment (transplanting date:17 January 2020, removal date: 23 September 2020) using varieties Gina (red) and Kathia (orange). All the varieties were sourced from Syngenta Australia (Macquarie Park, NSW, Australia).

2.2 Description of the glasshouse facility, LBF film characteristics, and experimental design

The NVPCC was established jointly by Western Sydney University and Horticulture Innovation Australia in 2017 at the Western Sydney University Hawkesbury Campus, Richmond, NSW, Australia (latitude: −33.611692° S, longitude: 150.745281° E). The NVPCC utilizes an 1,800 m^2 high-tech autonomous hydroponic glasshouse, based on facilities designed in the Netherlands, that is environmentally controlled by Priva software and hardware (Priva, De Leir, The Netherlands). It was established as a research, education, and training facility to address the most pressing horticultural research questions and train emerging leaders in the Australian PC industry.

The LBF experiment used four 105 m^2 glasshouse research compartments. All the research compartment roofs were fitted with HD1AR 70% hazed glass, and the walls were fitted with tempered clear glass. Two of these compartments were used as the controls. The treatment (LBF) compartments had an LBF film, which is a ULR-80 window film (Solar Gard, Saint-Gobain Performance Plastics, Sydney, NSW, Australia) designed for office buildings to reduce incoming sunlight and energy used to cool the building. The manufacturer states that the film blocks spectral light in varying amounts as follows: ~88% infrared and far-infrared light from 780 nm–2,500 nm and >99% of ultraviolet (UV) light from 300 nm–400 nm. Overall, LBF blocks 43% of the total solar energy while allowing 40% transmission, 54% absorption, and 6% reflection. The film was applied to the ceiling, side walls, entry walls, and shared interior walls of the treatment compartments. Because of the infrastructure of the mechanical coolers set at the entry of each treatment compartment, LBF was not applied to the three ceiling panels per compartment and the top eave panels of each entry wall.

Two trials of two *C. annuum* varieties were grown under the LBF treatment and control. The first trial began in autumn and is denoted as AE (Autumn Experiment) herein. The second trial began in the summer and was denoted as SE (Summer Experiment). Capsicum seedlings were transplanted into 1 m-long Grodan Grotop Expert rockwool slabs (Roermond, Limburg, The Netherlands) with four plants per slab in the control and LBF compartments. Each gutter contained 10 slabs, for a total of 240 plants per room. Two weeks after transplantation, two stems from each plant were selected and trellised onto plastic strings supported by a high-wire system. Plants were grown according to commercial practices of hydroponic production of vegetables in greenhouses under non-limiting water and nutrient (EC: 2.5 dS m^{-1}–3.0 dS m^{-1}, pH: 5.0–5.5) conditions at [CO_2] (489.6 μl l^{-1} and 476.6 μl l^{-1} daytime average), temperature (25.3/19.3 and 25.2/19.3°C day/night average), RH (74.2/72.9% and 74.2/77.5%, day/night average) and natural light for AE and SE, respectively (He et al., 2022). The environmental variables, including temperature, relative humidity, and CO_2 concentration at canopy level, were monitored in all glasshouse compartments at 5-minute intervals. Data were stored using the Priva system.

2.3 Light quantity and quality measurements

A huge array of light sensors is available to characterise and quantify the spectrum of light received by plants. PAR sensors measure photon flux density in photons m^{-2} s^{-1} from 400 nm to 700 nm. Net radiometers measure incoming and outgoing LW and SW radiation in W m^{-2}, and while SW and LW bandwidths differ slightly between instrument models, in general the bandwidth is 350 nm–2,500 nm for SW radiation and 2,500 nm–50,000 nm for LW radiation. Net radiometers were designed to measure the energy balance of a system and are thus important instruments for understanding energy fluxes (Mauder et al., 2020).

One major issue with most PAR sensors on the market is that they are usually calibrated for open-sky broad-spectrum solar radiation from 400 nm to 700 nm, and do not reflect PAR from monochromatic, supplemental, or spectrally altered light sources. Although PAR sensors measure total light from 400 nm to 700 nm they do not measure individual wavebands. Therefore, a spectroradiometer is necessary to understand the spectra available to the plant. Spectroradiometers measure instantaneous quantities of photons from higher resolution bandwidths (2 nm–15 nm resolution), usually within the 300 nm–1,200 nm range, and sometimes up to 2,500 nm, as is the case for the ASD FieldSpec (Malvern Panalytical, Malvern, Worcestershire, UK). The advantage of these sensors is that they measure the quantity of light of each wavelength incident on the crop, and these data can be transformed into PAR for comparability.

In August 2018, light sensor arrays were installed to characterize the light environment in both the control and LBF research rooms. These were connected to CR1000X data loggers (Campbell Scientific Inc., Logan, UT, USA) and programmed to measure continuously at 5-minute intervals. Each research room contained a PAR sensor (LI-190SZ Quantum Sensor, LI-COR, Lincoln, NE, USA), which measures photons from 400 nm to 700 nm, at the top of each bay. The incoming and outgoing SW radiation, and LW radiation were measured at the top of each glasshouse room using a net radiometer (SN-500, Apogee Instruments Inc., Logan, UT, USA). Using this net radiometer, the energy balance of each compartment was calculated, which is critical for understanding the impact of LBF on the light environment for biological responses as well as for cooling and heating energy use required to maintain optimal temperatures throughout the plant growth and production cycle. Diffuse PAR radiation was measured in one control room and one LBF treatment room using a diffuse light sensor (BF5 sunshine sensor; Delta T Devices, Burwell, Cambridge, UK). Variation in PAR incident on the crop canopy was measured by PAR sensors (LI-190R-SMV-50 Quantum Sensor, LI-COR, Lincoln, NE, USA) positioned at a maximum of 50 cm above the crop canopy and raised intermittently before being obscured by the growing plants. See Table 1 for technical information and the position of the light sensor.

2.4 Light penetration measurements

To understand how light penetrated the crop canopy across the control and LBF, measurements were taken above the canopy, halfway down the canopy within the region of canopy growth (not the aisle), and at the base of the plant. Five measurements were taken at each canopy level at three positions along the gutter length of plants 5, 20, and 35 (Figure 1). These measurements were averaged per height for the LBF and control treatments.

2.5 Growth, yield, and biomass measurements

Growth was measured weekly, after two stems were selected from each plant. Plant growth rate was defined as the stem elongation rate in cm d^{-1}. To measure this, the string supporting the stem of the plant was marked on the apical meristem of the stem. The length from the previous week's mark to the current week's mark was recorded. Pruning was conducted every two weeks. The pruned biomass was collected from 40 stems per variety. The material was dried at 70°C for a minimum of three days and weighed directly after cooling to room temperature. Counts of buds, flowers, and fruit (per 20 stems per variety per room) were conducted every two weeks before each scheduled pruning to assess differences in bud and flower presence and fruit carrying capacity across treatments.

Harvests of the capsicum crop were done weekly once fruits had ripened (90%–100% color change), and fruit number as well as individual weight were recorded for 40 stems per variety per room. The fruits were visually graded as follows: 1 if the fruit had a perfect shape, color, and shine; 2 if the fruit had a perfect color and shine but not a perfect shape; 3 if the fruit was misshapen with potentially some blemishes; and 4 if the fruit was tiny, diseased, and/or not edible. Grades 1 and 2 were selected to assess marketable yield and fruit number per plant.

Mature fruits, selected based on the color (red and orange) of individual fruits, were harvested, and the individual fruit weight and number of fruits per stem were recorded every week. The fruits were graded as marketable (≥100 g, including the extra-large fruit ≥250 g) and unmarketable, which included small (<100 g, edible) fruits and fruits with rotting, cracking, lobing, and other deformities.

2.6 Data analysis and statistics

All raw biological data were collated in Excel (version 2204, Microsoft, Redmond, WA, USA), and continuous environmental data were logged into CSV files and automatically saved. All data analyses were performed using the R software (R Core Team, 2021). PAR measurements were converted into daily light integrals (DLI; mol photons m^{-2} d^{-1}) according to Poorter et al. (2019). Statistical analyses were performed following Chavan et al. (2020) because the

TABLE 1 Light sensor array description with associated technical data and position within the LBF and control compartments used across both AE and SE.

Sensor Type	Spectral Range	Units	Position in room	Trait
PAR	Incoming 400 nm–700 nm PAR/Visible	µmol m^{-2} s^{-1}	1—Southwest 2—South mid 3—Southeast 4—Northwest 5—Northeast 6 – top of room	Photosynthesis
Diffuse Light	Incoming 400 nm–700 nm PAR/Visible + Diffuse Fraction	W m^{-2}	1—top of room (1 LBF room and 1 C only)	Photosynthesis
Net Radiometer	Incoming + outgoing SW 295 nm–2685 nm UV, Visible, NIR LW 5,000 nm–30,000 nm Infrared and Far Infrared	W m^{-2}	1—top of room	Energy balance
Spectroradiometer	Incoming 300 nm–1,100 nm UV, PAR, NIR	W m^{-2}	1—handheld used to assess light penetration throughout canopy	Light quality

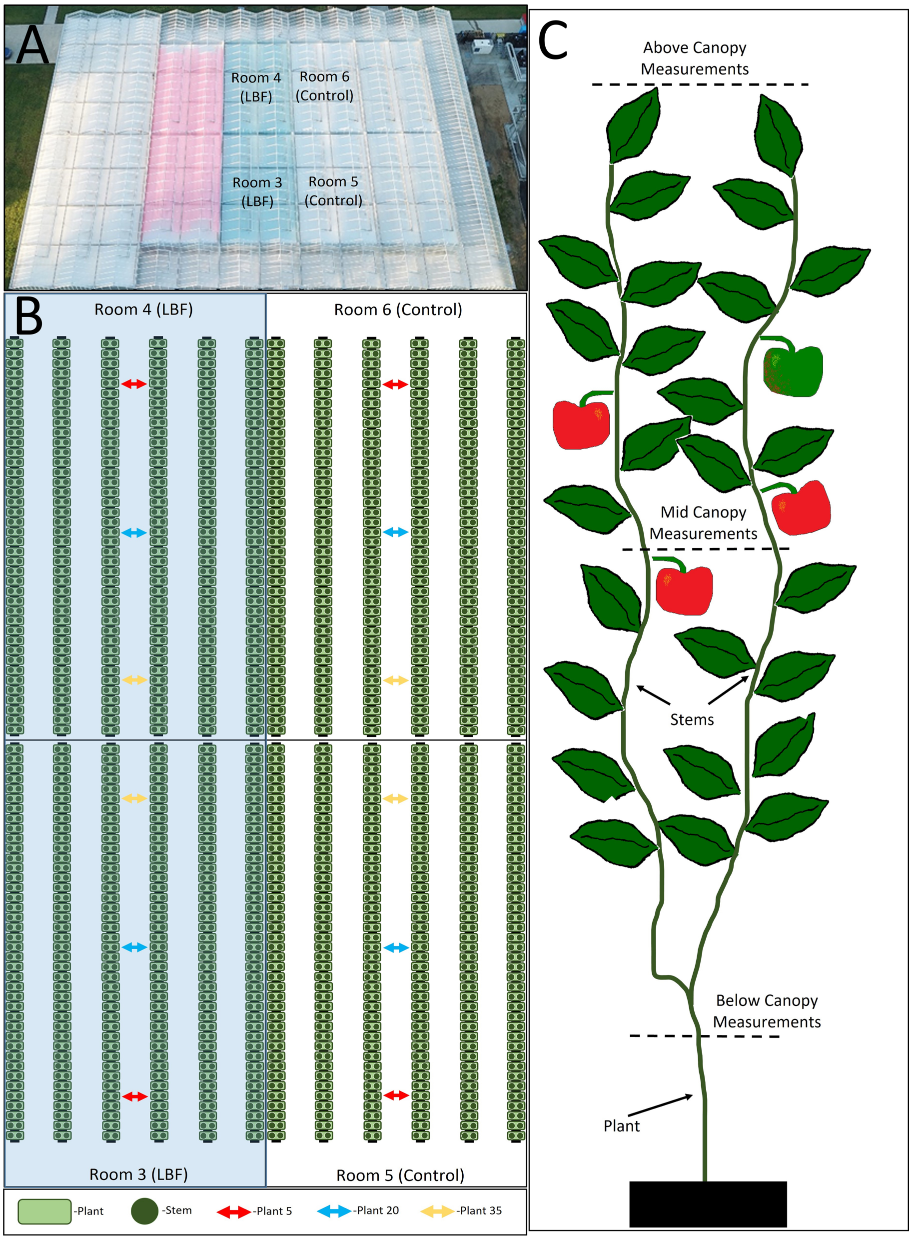

FIGURE 1
Diagram of glasshouse rooms and light penetration position measurements. Aerial view of the glasshouse with labeled research rooms **(A)**. Room diagram with indicated positions of plants 5, 20, and 35 where the light penetration measurements were performed **(B)**. Locations within the canopy light measurements were completed **(C)**.

crop measurement data and experimental design were similar. All statistical tests were performed using the R statistical package. The Shapiro–Wilks method was applied to verify whether data were normally distributed, and Bartlett's test was used to verify the equality of variances. Once data were confirmed to be normally distributed with equal variance, one-way or two-way Analysis of Variance (ANOVA) was used. The Kruskal–Wallis Test was used when the data were not normally distributed but had equal variance. Welch's ANOVA was used for normally distributed data with unequal variance. The p-values are either mentioned as values or as significance levels indicated as "*" (p-value <0.05), "**" (p-value <0.01) and "***" (p-value <0.001).

3 Results

3.1 LBF reduces PAR with a greater impact during high solar radiation conditions in both AE and SE

The PAR sensors positioned at the top of each glasshouse room showed a consistent reduction in PAR across both AE and SE for LBF compared with the control. During AE, LBF reduced the mean DLI by 27% and cumulative DLI by 24% (Figure 2A), while during SE, LBF reduced the mean DLI by 28% and cumulative DLI by 27% (Figure 2B). However, for the canopy-level PAR sensors, the

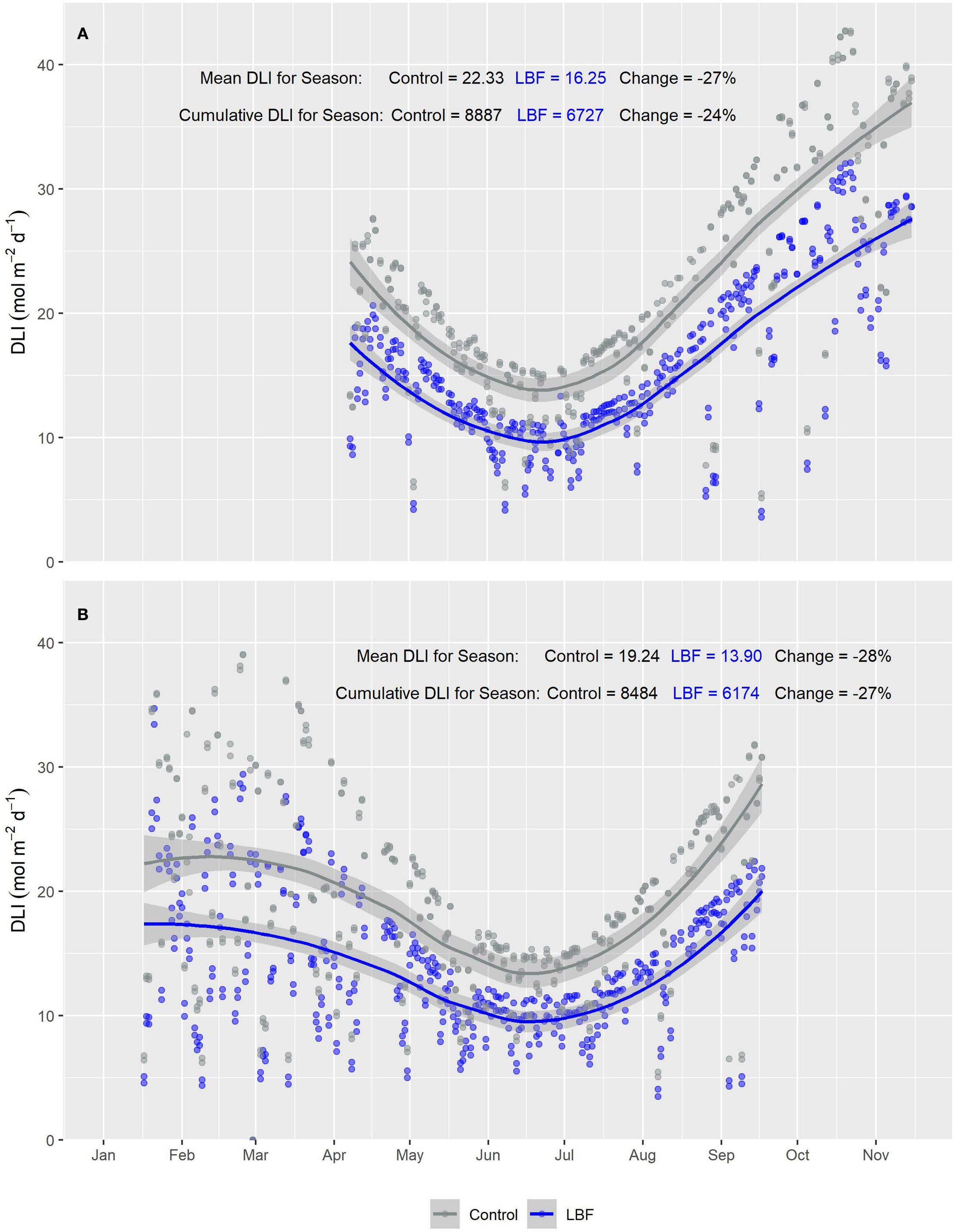

FIGURE 2
Smooth plot of the average cumulative daily light integral (DLI; mol m^{-2} d^{-1}) over time for roof-level PAR sensors for both LBF and control for **(A)** Autumn Experiment (AE) and **(B)** Summer Experiment (SE). Each data point represents the average DLI for a single day. The blue (LBF) and gray (control) lines are fitted loess curves with formula y ~ x and shaded regions represent the 95% confidence interval.

difference in PAR between the control and LBF was observed only in the high sun angle months of summer and when the canopy PAR sensors were at higher height positions throughout the seasons. Mean and cumulative reductions by LBF were observed for canopy level PAR sensors for both AE and SE with a cumulative season reduction in DLI of 14% and a mean season reduction in DLI of 18% for AE and a cumulative season reduction in DLI of 21% and a mean season reduction in DLI of 21% for SE (Figures 3A, AE and B, SE). In AE, there was no significant PAR reduction for the first ~4 months of the season, while the remaining ~3 months of the season had significant reductions in DLI due to LBF. In SE, there was a significant reduction in PAR from transplanting for ~3.5 months, followed by ~2.5 months during winter with no significant reduction in DLI due to LBF, while the last ~1.5 months of the

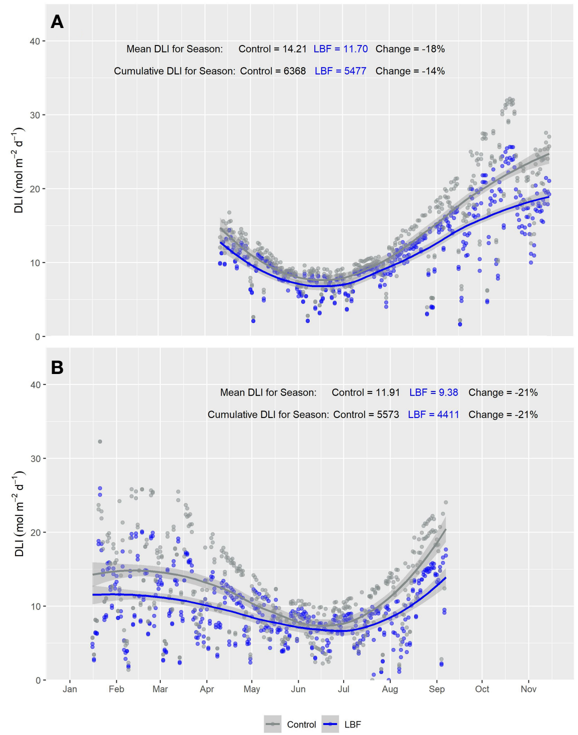

FIGURE 3
Smooth plot of cumulative daily light integral (DLI; mol m^{-2} d^{-1}) over time across five canopy-level PAR sensors for both LBF and control treatments for **(A)** Autumn Experiment (AE) and **(B)** Summer Experiment (SE). Each data point represents the DLI for a single day across the five canopy-level PAR sensors. The curves are fitted with loess using the formula y ~ x. Blue (LBF) and gray (control) lines are fitted loess curves with formula y ~ x and shaded regions represent the 95% confidence interval.

crop showed a significant LBF PAR reduction. Although AE and SE were roughly the same length, ~57% of the AE season's growth was without light reduction due to LBF, whereas only ~33% of the SE season's growth was without light reduction due to LBF.

3.2 PAR and net radiation are different at solar transition periods throughout AE and SE

Given that LBF reduces canopy PAR differentially throughout the year, it is important to understand how it impacts other light regions and the daily sums of PAR during solar transition periods. Solar azimuth, which is the location of sunrise and sunset on the horizon, and solar altitude, which is the height of the sun from the horizon, transition during the year with impacts on the length of the photoperiod (Bowen, 1979). These solar transition periods around the equinoxes and solstices may affect the light differentials produced by LBF and further influence the physiological responses of the crop.

The incoming SW radiation was significantly reduced (53%–58%) by LBF across solar transition periods and seasons (AE and SE). The largest reductions in incoming SW radiation by LBF for AE and SE were 60% and 58%, respectively, which occurred during the winter solstice in both seasons. The smallest reductions in incoming SW radiation by LBF for AE and SE were 54% and 52%, respectively, which occurred at the beginning of each experiment in both seasons. Although these reductions vary, the reduction in incoming SW radiation is relatively consistent across solar transition periods and seasons. However, the reduction due to LBF on outgoing SW radiation varied more than the incoming SW radiation, ranging from 31% to 70% across both seasons and solar transition periods. The largest reductions in outgoing SW radiation were 69% for AE at the summer solstice and 70% for SE at the Spring equinox, both of which occurred at the end of each crop cycle (Table 2).

Interestingly, LBF increased incoming LW radiation by 3%–8% across both seasons. The smallest increases in incoming LW radiation were 3% for AE and 4% for SE, which occurred during the winter solstice in both seasons. Larger increases in incoming LW radiation occurred at the spring equinox for AE with an 8% increase, while the start of the season, the autumn equinox, and spring equinox all showed a 7% increase by LBF for SE. Outgoing LW radiation was not impacted by LBF (Table 2).

The LBF affected incoming and outgoing SW and LW radiation, as well as net SW (incoming SW − outgoing SW), net LW (incoming LW − outgoing LW), and net radiation (net SW + net LW). Net SW radiation was reduced by LBF across AE and SE and solar transition periods by 52%–60%, with the largest reductions occurring at the winter solstices for both AE and SE, with observed reductions of 60% and 58%, respectively (Table 3). LBF increased the net LW radiation by 47%–480% across both the AE and SE and solar transition periods. The smallest increases in net LW occurred during the winter solstice for both AE and SE; for these periods, net LW was negative. However, for all other solar transition periods for AE and SE, LBF caused net radiation to be positive. The largest increase in net LW was 371% during the summer solstice for AE and 480% during the spring equinox for SE; both periods were at the end of the crop season. Overall, LBF reduced net radiation across AE and SE and solar transition periods by 36%–66%, with the highest reductions in net radiation occurring on the winter solstices for both AE and SE, with reductions of 66% and 47%, respectively.

While roof-level PAR showed a consistent reduction in DLI across both AE and SE, canopy-level PAR sensors only showed a significant reduction in DLI during the high light period when comparing data using a smoothed plot representation. However, when comparing full sun daily sums during solar transition periods, LBF reduced DLI significantly, and this reduction seems to be influenced by the height of the canopy PAR sensors (Table 4). LBF reduced the canopy level DLI by 8%–25.3%, with a span of 17.3%, across solar transition periods and seasons. The largest reduction in AE was 23.3% in the summer solstice and 25.3% in the SE at the autumn equinox. Roof-level PAR sensors showed a more consistent reduction in DLI across solar transition periods, for both AE and SE, with a span of 4.2%, ranging from 26.0% to 30.2%.

3.3 LBF film increases diffuse light conditions through AE and SE

The daily average proportion of diffuse light was increased by LBF in AE (Figure 4A) and SE (Figure 4B). In AE, the average daily

TABLE 2 Incoming and outgoing SW and LW radiation for the start of the Autumn Experiment (AE) and the Summer Experiment (SE) and following solar transitions through which each crop was grown. Not significant p-values are indicated as "NS".

			Incoming SW					Outgoing SW				
Time of Year	Date	DAT	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value
2019 Start AE	17-04-19	12	3.81 ± 0.07	1.73 ± 0.03	−2.08	−55	***	0.33 ± 0.01	0.23 ± 0.01	−0.10	−31	***
2019 Winter Solstice	19-06-19	75	2.33 ± 0.01	0.98 ± 0.01	−1.35	−58	***	0.16 ± 0.01	0.11 ± 0.01	−0.05	−31	***
2019 Spring Equinox	20-09-19	168	5.19 ± 0.14	2.30 ± 0.05	−2.89	−56	***	0.47 ± 0.02	0.22 ± 0.02	−0.26	−54	***
2019 Summer Solstice	29-11-19	238	6.15 ± 0.11	2.70 ± 0.06	−3.46	−56	***	0.76 ± 0.02	0.23 ± 0.01	−0.53	−69	***
2020 Start SE	01-03-20	44	5.40 ± 0.04	2.56 ± 0.03	−2.84	−53	***	0.48 ± 0.01	0.21 ± 0.01	−0.27	−56	***
2020 Autumn Equinox	19-03-20	62	4.95 ± 0.11	2.30 ± 0.05	−2.65	−54	***	0.43 ± 0.01	0.17 ± 0.02	−0.26	−60	***
2020 Winter Solstice	19-06-20	154	2.25 ± 0.01	0.96 ± 0.01	−1.29	−57	***	0.18 ± 0.01	0.09 ± 0.01	−0.09	−52	***
2020 Spring Equinox	15-09-20	242	4.67 ± 0.05	2.10 ± 0.02	−2.57	−55	***	0.56 ± 0.01	0.17 ± 0.01	−0.39	−70	***

			Incoming LW					Outgoing LW				
Time of Year	Date	DAT	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value
2019 Start AE	17-04-19	12	10.20 ± 0.03	10.67 ± 0.04	0.47	+ 5	***	10.60 ± 0.02	10.55 ± 0.02	−0.05	−1	.
2019 Winter Solstice	19-06-19	75	9.59 ± 0.06	9.83 ± 0.03	0.24	+ 3	**	10.24 ± 0.02	10.18 ± 0.02	−0.06	−1	NS
2019 Spring Equinox	20-09-19	168	9.93 ± 0.04	10.63 ± 0.06	0.69	+ 7	***	10.42 ± 0.01	10.41 ± 0.02	−0.01	0	NS
2019 Summer Solstice	29-11-19	238	10.33 ± 0.04	11.19 ± 0.08	0.86	+ 8	***	10.58 ± 0.02	10.53 ± 0.02	−0.05	0	NS

(Continued)

TABLE 2 Continued

Time of Year	Date	DAT	Incoming LW					Outgoing LW				
			Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value
2020 Start SE	01-03-20	44	10.57 ± 0.14	11.33 ± 0.12	0.76	+ 7	**	10.84 ± 0.10	10.69 ± 0.07	−0.16	−1	NS
2020 Autumn Equinox	19-03-20	62	10.39 ± 0.09	11.15 ± 0.09	0.76	+ 7	***	10.62 ± 0.04	10.52 ± 0.04	-0.10	-1	NS
2020 Winter Solstice	19-06-20	154	9.71 ± 0.06	10.09 ± 0.02	0.39	+ 4	***	10.11 ± 0.02	10.10 ± 0.01	-0.01	0	NS
2020 Spring Equinox	15-09-20	242	10.16 ± 0.10	10.85 ± 0.07	0.69	+ 7	***	10.31 ± 0.03	10.30 ± 0.02	-0.01	0	NS

Data are average daily sums across three full sun days around each respective Date ± standard error of the mean (n = 6) and statistical analysis was performed using parametric or non-parametric analyses (one-way analysis of variance (OA), Kruskal–Wallis (KW), or Welch's ANOVA (WA). The p-values significance levels indicated as "**" (p-value <0.01), and "***" (p-value <0.001).

diffuse light fractions in the control and LBF groups were 47.0% and 54.5%, respectively. In SE, the average daily diffuse light fractions were 55.3% and 64.2% in the control and LBF groups, respectively. Although LBF generated a higher diffuse fraction of light, the total diffuse light received was significantly less than that of the control, except from June to mid-August for AE (Figure 5A) and from May to September for SE when there was no impact (Figure 5B).

3.4 LBF significantly reduces far-red light throughout canopy profile

Diffuse light penetrates deeper into the canopy than direct light, potentially improving photosynthesis in mid- and bottom-canopy leaves (Babla et al., 2020). Above-canopy light was higher overall for the control than for LBF (Figure 6A), with a significant reduction of 60% in far-red light (P = 0.004, Table 5). Minimal light was transmitted to mid-canopy (Figure 6B) and low-canopy (Figure 6C) heights in the blue, green, red, and PAR spectral regions with no significant difference between LBF and control; however, LBF significantly reduced far-red light for mid-canopy and low-canopy positions by 57% (P = 0.002) and 64% ($P = 1.01 \times 10^{-5}$), respectively (Table 5).

3.5 Plant morphological responses to LBF and seasons

Plant growth rate, number of buds, flowers, developing fruit, yield (fruit mass per plant and fruit number per plant), and pruned biomass were measured in both experiments (Table 6). There was no difference in growth rate across LBF and the control for the red variety in AE and both varieties in SE; however, the growth rate was significantly higher in SE than in AE (red variety, $P = 4.7 \times 10^{-14}$ and orange variety, $P = 2 \times 10^{-16}$). The growth rate was slightly increased by LBF in the orange variety of AE. During AE, LBF the increased numbers of buds for both red and orange capsicum by 11.2% (P = 0.006) and 16.4% ($P = 1.9 \times 10^{-4}$), flowers for both red and orange varieties by 16.1% ($P = 4.9 \times 10^{-4}$) and 13.8% ($P = 4.0 \times 10^{-4}$), respectively, and developing fruit for the orange variety only by 8% (P = 0.015). There was no treatment effect for buds, flowers, or developing fruit in SE; however, SE plants produced more buds, flowers, and developing fruit than in AE. There was significantly more pruned biomass in the AE season than in the SE for both varieties (red: $P = 7.4 \times 10^{-9}$ and orange: $P = 3.5 \times 10^{-6}$); however, there was no difference in pruned biomass across the control and LBF for either AE or SE.

3.6 LBF mainly reduces fruit yield in SE and not in AE

The total fruit yield per m^2 per year was higher for AE than for SE for both varieties (Table 6). During AE, the red variety produced 12.55 kg m^{-2} in control and 11.95 kg m^{-2} in LBF, and the orange variety produced 10.98 kg m^{-2} in control and 11.21 kg m^{-2} in LBF.

TABLE 3 Net SW, net LW and net radiation for the start of the Autumn Experiment (AE) and the Summer Experiment (SE) and following solar transitions through which each crop was grown.

Net SW							
Time of Year	Date	DAT	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value
2019 Start AE	17-04-19	12	3.49 ± 0.06	1.51 ± 0.03	−1.98	−57	***
2019 Winter Solstice	19-06-19	75	2.17 ± 0.02	0.87 ± 0.01	−1.30	−60	***
2019 Spring Equinox	20-09-19	168	4.72 ± 0.13	2.08 ± 0.05	−2.63	−56	***
2019 Summer Solstice	29-11-19	238	5.39 ± 0.10	2.46 ± 0.06	−2.93	−54	***
2020 Start SE	01-03-20	44	4.92 ± 0.05	2.35 ± 0.02	−2.57	−52	***
2020 Autumn Equinox	19-03-20	62	4.52 ± 0.10	2.12 ± 0.04	−2.39	−53	***
2020 Winter Solstice	19-06-20	154	2.06 ± 0.02	0.87 ± 0.01	−1.19	−58	***
2020 Spring Equinox	15-09-20	242	4.11 ± 0.05	1.93 ± 0.01	−2.18	−53	***
Net LW							
Time of Year	Date	DAT	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value
2019 Start AE	17-04-19	12	−0.40 ± 0.02	0.12 ± 0.03	0.52	+ 131	***
2019 Winter Solstice	19-06-19	75	−0.65 ± 0.03	−0.35 ± 0.02	0.31	+ 47	***
2019 Spring Equinox	20-09-19	168	−0.49 ± 0.03	0.22 ± 0.04	0.71	+ 145	***
2019 Summer Solstice	29-11-19	238	−0.24 ± 0.03	0.66 ± 0.07	0.91	+ 371	***
2020 Start SE	01-03-20	44	−0.27 ± 0.04	0.64 ± 0.05	0.92	+ 334	***
2020 Autumn Equinox	19-03-20	62	−0.23 ± 0.05	0.62 ± 0.05	0.86	+ 368	***
2020 Winter Solstice	19-06-20	154	−0.40 ± 0.05	−0.01 ± 0.01	0.40	+ 99	***
2020 Spring Equinox	15-09-20	242	−0.14 ± 0.07	0.55 ± 0.06	0.69	+ 480	***
Net Radiation							
Time of Year	Date	DAT	Control (kWh m^{-2} day^{-1})	LBF (kWh m^{-2} day^{-1})	Δ (kWh m^{-2} day^{-1})	Change (%)	*p*-Value
2019 Start AE	17-04-19	12	3.09 ± 0.08	1.63 ± 0.05	−1.45	−47	***
2019 Winter Solstice	19-06-19	75	1.52 ± 0.04	0.52 ± 0.03	−0.99	−66	***
2019 Spring Equinox	20-09-19	168	4.23 ± 0.11	2.30 ± 0.06	−1.93	−46	***
2019 Summer Solstice	29-11-19	238	5.15 ± 0.10	3.13 ± 0.10	−2.02	−39	***
2020 Start SE	01-03-20	44	4.64 ± 0.07	2.99 ± 0.07	−1.65	−36	***
2020 Autumn Equinox	19-03-20	62	4.28 ± 0.06	2.75 ± 0.05	−1.54	-36	***
2020 Winter Solstice	19-06-20	154	1.61 ± 0.05	0.86 ± 0.01	−0.75	−47	***
2020 Spring Equinox	15-09-20	242	3.97 ± 0.09	2.48 ± 0.07	−1.49	−37	***

Data are average daily sums across three full sun days around each respective Date ± standard error of the mean (n = 6) and statistical analysis was performed using parametric or non-parametric analyses (one-way analysis of variance (OA), Kruskal–Wallis (KW), or Welch's ANOVA (WA). Significance indications are stated in the methodology section.

During SE, the red variety produced 12.00 kg m^{-2} in control and 8.47 kg m^{-2} in LBF, and the orange variety produced 10.31 kg m^{-2} in control and 8.59 kg m^{-2} in LBF. LBF caused a slight increase in yield for the orange variety with an AE of 2.1%; however, in SE, LBF produced a 16.7% reduction in the orange variety. LBF significantly reduced plant fruit numbers for the red variety for both AE and SE by 15% ($P = 0.003$) and 31.5% ($P = 1.1 \times 10^{-9}$), respectively. The fruit number per plant for the orange variety showed no difference across LBF and control for AE; however, there was an 18.1% reduction in fruit number in LBF in comparison to the control in SE ($P = 6.4 \times 10^{-15}$). When comparing marketable yield mass, LBF increased the orange variety by only 8.4% ($P = 0.016$), while the read variety was unaffected. However, marketable fruit numbers were not affected by LBF in AE. In SE, the yield mass was decreased by LBF for red and orange by 29.3% ($P = 1.68 \times 10^{-8}$) and 17.5% ($P = 5.75 \times 10^{-8}$), respectively. Marketable fruit number was also

TABLE 4 DLI (mol m–2 day–1) of photosynthetically active radiation average across canopy and roof level PAR sensors for the beginning of each experiment and solar transition periods for the Autumn Experiment (AE) and the Summer Experiment (SE).

PAR at Canopy							
Time of Year	Date	DAT	Control (mol m^{-2} day^{-1})	LBF (mol m^{-2} day^{-1})	Δ (mol m^{-2} day^{-1})	Change (%)	*p*-Value
2019 Beginning of AE	17-04-19	12	15.4 ± 0.4	13.6 ± 0.2	−1.7	−11.2	**
2019 Winter Solstice	19-06-19	75	8.8 ± 0.2	8.1 ± 0	−0.7	−8.0	**
2019 Spring Equinox	20-09-19	168	22.2 ± 0.8	18.6 ± 0.5	−3.5	−15.8	**
2019 Summer Solstice	29-11-19	238	26.7 ± 0.8	20.5 ± 0.7	−6.2	−23.3	***
2020 Beginning of SE	01-03-20	44	25.2 ± 0.2	19.3 ± 0.2	−5.9	−23.4	***
2020 Autumn Equinox	19-03-20	62	22.4 ± 0.4	16.7 ± 0.2	−5.7	−25.3	***
2020 Winter Solstice	28-06-20	154	9.9 ± 0.2	8.2 ± 0.2	−1.7	−16.7	***
2020 Spring Equinox	15-09-20	242	0.2 ± 0	0.3 ± 0	0.1	53.7	***
PAR at Roof							
Time of Year	Date	DAT	Control (mol m^{-2} day^{-1})	LBF (mol m^{-2} day^{-1})	Δ (mol m^{-2} day^{-1})	Change (%)	*p*-Value
2019 Beginning of AE	17-04-19	12	26.8 ± 0.3	19.5 ± 0.3	−7.3	−27.2	***
2019 Winter Solstice	19-06-19	75	15.2 ± 0.1	10.8 ± 0.1	−4.4	−29.2	***
2019 Spring Equinox	20-09-19	168	34.6 ± 0.7	25.3 ± 0.6	−9.3	−26.9	***
2019 Summer Solstice	29-11-19	238	39.5 ± 0.9	29.1 ± 0.7	−10.4	−26.3	***
2020 Beginning of SE	01-03-20	44	38 ± 0.4	28.1 ± 0.3	−9.9	−26.0	***
2020 Autumn Equinox	19-03-20	62	33.8 ± 0.6	24.7 ± 0.5	−9.2	−27.1	***
2020 Winter Solstice	28-06-20	154	14.9 ± 0.1	10.6 ± 0.3	−4.3	−28.7	***
2020 Spring Equinox	15-09-20	242	31.1 ± 0.2	21.7 ± 0.2	−9.4	−30.2	***

Data are average daily sums across three full sun days around each respective Date ± standard error of the mean (n = 6) and statistical analysis was performed using parametric or non-parametric analyses (one-way analysis of variance (OA), Kruskal–Wallis (KW), or Welch's ANOVA (WA). Significance indications are stated in the methodology section.

reduced by LBF by similar proportions as mass, with a reduction in the red variety of 29.3% ($P = 1.33 \times 10^{-5}$) and a reduction in the orange variety of 17.4% (P = 0.002).

4 Discussion

This experimental section provided an analysis of the impact of LBF on light quantity and quality throughout two crop cycles of *C. annuum* planted in the autumn of 2019 (AE) and in the summer of 2020 (SE) and the subsequent biological responses of growth, biomass, and fruit yield. Overall, the impact of LBF and planting time differentially affected crop growth and productivity.

4.1 Impacts of LBF on light environment across all installed light sensors

While the smoothed plot for canopy-level PAR sensors did not show an impact from LBF during winter months and during lower sensor height positions for AE and SE, the sums of full sun days around the winter solstice showed a significant reduction in LBF compared to the control. When full sun days were compared, canopy level PAR was reduced differentially depending on the time of year for both AE and SE, ranging from 8% to 25.3%, whereas roof level reduction remained constant (26.0%–30.2%) regardless of the time of year. This suggests that the canopy light environment is dependent on crop height. Structural shading impacts light measurements such that higher canopy positions have less shading overall than lower positions, particularly during winter months when shadows are longer. Inside a glasshouse, structural shading can reduce light by up to 30% (Gruda, 2005). Differences in the structural components and the directional position of the glasshouse may cause specific light sensors to be more shaded than others, even though they are in the same position in each room. PAR reduction differences may also occur over the year because some of the glass panels on the wall over the door and on the roof close to the eaves could not be covered with LBF owing to infrastructure. As the sun angle decreases during the winter months, light begins to enter the room from positions that do not

TABLE 5 Light region average sums for each position throughout the canopy.

Above Canopy					
Wavelength (nm)	Control (µmol m^{-2} s^{-1})	LBF (µmol m^{-2} s^{-1})	Δ (µmol m^{-2} s^{-1})	Change (%)	*p*-value
Blue (340 nm–499 nm)	676.2 ± 76.9	523.0 ± 44.7	−153	−22.7	0.078
Green (500 nm–599 nm)	732.2 ± 92.5	612.1 ± 49.5	−120.00	−16.4	0.109
Red (600 nm–699 nm)	726.7 ± 97.0	524.1 ± 55.3	−203	−27.9	0.078
Far-red (719 nm–850 nm)	834.5 ± 107.5	331.8 ± 77.5	−503	−60.2	0.007
PAR (380 nm–699 nm)	2085.6 ± 262.0	1652.8 ± 145.2	−433	−20.8	0.078
Mid Canopy					
Wavelength (nm)	Control (µmol m^{-2} s^{-1})	LBF (µmol m^{-2} s^{-1})	Δ (µmol m^{-2} s^{-1})	Change (%)	*p*-value
Blue (340 nm–499 nm)	40.3 ± 11.8	32.9 ± 12.3	−7	−18.3	0.262
Green (500 nm–599 nm)	48.3 ± 14.7	44.7 ± 15.7	−4	−7.5	0.337
Red (600 nm–699 nm)	43.2 ± 15.4	36.3 ± 14.0	−7	−16.1	0.200
Far-red (719 nm–850 nm)	353.7 ± 30.6	153.7 ± 38.2	−200	−56.5	0.016
PAR (380 nm–699 nm)	128.8 ± 41.2	113.5 ± 41.8	−15	−11.9	0.262
Bottom Canopy					
Wavelength (nm)	Control (µmol m^{-2} s^{-1})	LBF (µmol m^{-2} s^{-1})	Δ (µmol m^{-2} s^{-1})	Change (%)	*p*-value
Blue (340 nm–499 nm)	12.0 ± 2.7	11.7 ± 2.2	−0.3	−2.1	0.943
Green (500 nm–599 nm)	16.6 ± 3.1	18.4 ± 2.9	1.8	11.1	0.749
Red (600 nm–699 nm)	13.9 ± 3.2	14.4 ± 2.9	0.6	4.1	0.631
Far-red (719 nm–850 nm)	246.4 ± 12.7	89.6 ± 14.5	−157.00	−63.7	0.004
PAR (380 nm–699 nm)	41.8 ± 8.7	44.6 ± 8.0	3	6.7	0.873

Data are mean ± standard error of the mean (n = 6) and statistical analysis was performed using a Kruskal–Wallis Test.
Far-red radiation is significantly reduced by LBF throughout the canopy.

have LBF applied, whereas with high sun angles in summer, light primarily enters from the roof, which is fully covered by LBF. Further investigation of canopy-level light differences across room areas with height would be useful.

Net radiation measurements are integral to understanding the energy balance of a system, and net radiometers are used in natural ecosystems, broad acre cropping, and PC agriculture (Koksal et al., 2018; Rebmann et al., 2018; Saadon et al., 2021). Introducing film

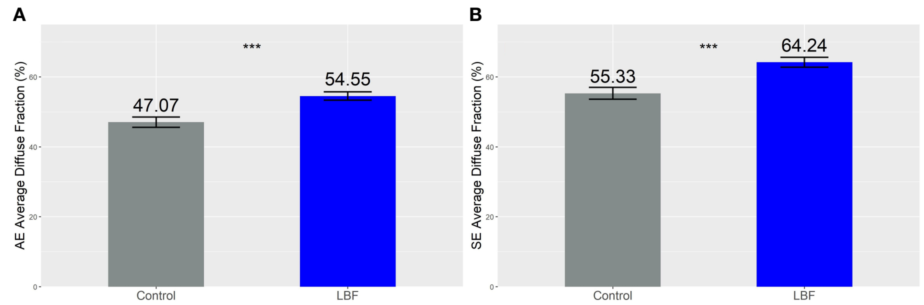

FIGURE 4
Average diffuse fraction (%) across the whole season for the **(A)** Autumn Experiment (AE) and **(B)** Summer Experiment (SE). The numbers above the bars are the diffuse light fraction (%), and the error bars are the standard error of the mean. Both seasons were significantly different across the control and LBF groups, *** indicates P <0.001 from a one-way analysis of variance.

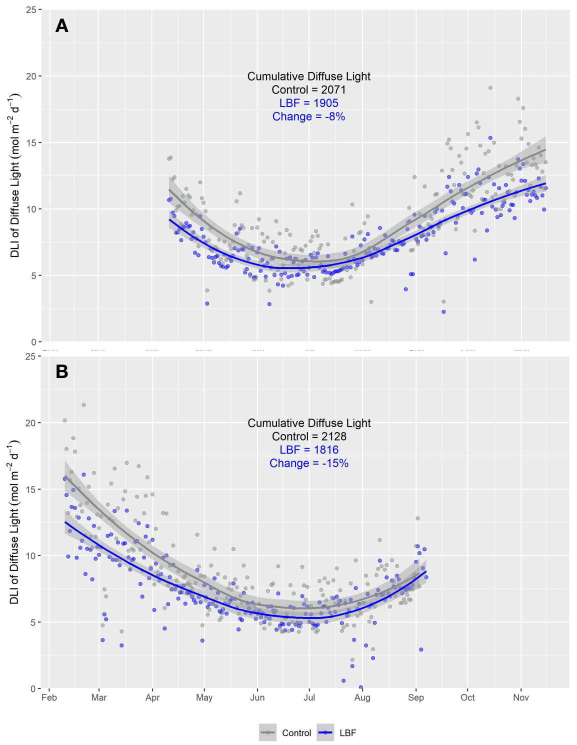

FIGURE 5
Smooth plot of the cumulative daily light integral (DLI; mol m^{-2} d^{-1}) of diffuse light over time for both LBF and control for **(A)** AE and **(B)** SE. Each data point represents the average DLI for a single day. Each data point represents the average DLI from five sensors for a single day. The blue (LBF) and gray (control) lines are fitted loess curves with formula y ~ x and shaded regions represent the 95% confidence interval.

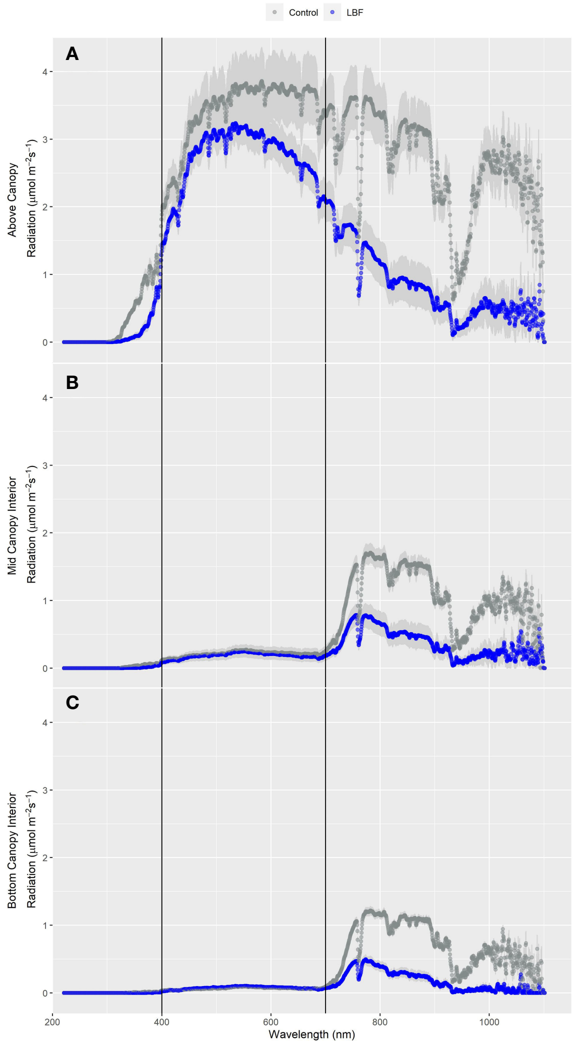

FIGURE 6
Light spectrum measured just above the canopy **(A)**, mid-height within the canopy **(B)**, and below the canopy **(C)** on 17-09-2020 for the SE crop during full sun conditions. For each spectrum, each point is the measured µmol/m^2/s every 0.5 nm across the 300 nm–1,100 nm sensor range. The shaded regions represent the standard error of the mean.

technology to a glasshouse that reflects and absorbs SW light will impact the energy balance of the crop and the requirements to maintain optimal crop temperatures. In this study, it was found that while incoming SW radiation was reduced relatively consistently throughout the year (53%–58%), the outgoing SW and incoming LW varied with solar transition periods, and outgoing LW was unimpacted by LBF. The largest reduction in outgoing SW radiation was at the end of the summer solstice for AE and the spring equinox for SE. During this period, the top canopy of the crop absorbs larger amounts of SW radiation, including PAR and some LW radiation, because the overall leaf area is highest at the end of the crop cycle (Rosati et al., 2001). The incoming LW radiation increased overall for LBF rooms, with the largest increase in the summer (7%) and the lowest increase in the winter (3%). The larger increase in incoming LW radiation during the summer was due to the LBF absorbing the blocked SW radiation as heat and then emitting it into the room. However, SW radiation accounts for the majority of energy incident to the glasshouse, and the overall net radiation across AE, SE, and solar transition periods was reduced by 36%–66% in both seasons. The LBF was designed for residential buildings with a vertical orientation to the sun and was not designed for rooftops. While a primarily horizontal orientation to the sun may facilitate heat absorption and negatively affect the cooling capacity of the rooms (Chaiyapinunt et al., 2005), the total amount of net radiation reduction by LBF should result in a reduced need for cooling to maintain optimal temperatures throughout high radiation months. To understand the energy balance of LBF and control better, temperature measurements of the roof, walls, and canopy at different heights are required.

Light penetration throughout the canopy is an important factor for growers because overall photosynthesis increases when more of the canopy profile is illuminated. Growers base planting density on light levels throughout the year, and planting density and diffuse light levels affect light transmission throughout the crop canopy (Jovicich et al., 2004; Marcelis et al., 2006). LBF increased the diffuse fraction of total PAR; however, total diffuse light was either not affected or slightly reduced by LBF. The impact of light transmission throughout the crop canopy profile was investigated, as the diffuse

TABLE 6 Summary of statistical analyses using one-way and two-way analysis of variance (ANOVA) for the LBF and experiment effect on growth and yield parameters.

Parameter	Exp	Red							Orange						
		Control	LBF	Change (%)	LBF *p*-value	LBF	Exp *p*-value	LBF*Exp	Control	LBF	Change (%)	LBF *p*-value	LBF	Exp *p*-value	LBF*Exp
Growth parameters															
Growth rate(cm day^{-1})	AE	0.83 ± 0.02	0.85 ± 0.02	2.4	0.605	0.515	**$<2 \times 10^{-16}$**	0.201	0.77 ± 0.02	0.83 ± 0.02	7.8	**0.015**	0.552	**$<2 \times 10^{-16}$**	**0.004**
	SE	1.53 ± 0.04	1.47 ± 0.03	−3.9	0.262				1.49 ± 0.04	1.39 ± 0.04	−0.7	0.06			
Buds(n/plant/week)	AE	10.90 ± 0.36	12.12 ± 0.25	11.2	**0.006**	**0.03**	**$<2 \times 10^{-16}$**	0.614	10.84 ± 0.26	12.62 ± 0.37	16.4	**1.9×10^{-4}**	**8.7×10^{-4}**	**$<2 \times 10^{-16}$**	0.207
	SE	17.42 ± 0.59	18.18 ± 0.53	4.4	0.342				17.47 ± 0.44	18.28 ± 0.43	4.6	0.189			
Flower(n/plant/week)	AE	1.37 ± 0.04	1.59 ± 0.04	16.1	**4.9×10^{-4}**	0.104	**$<2 \times 10^{-16}$**	**0.015**	1.67 ± 0.04	1.90 ± 0.05	13.8	**4.0×10^{-4}**	**0.009**	0.258	**0.022**
	SE	2.02 ± 0.06	1.97 ± 0.06	−2.5	0.623				1.83 ± 0.05	1.84 ± 0.05	0.5	0.821			
Developing Fruit (n/plant/week)	AE	6.40 ± 0.17	6.53 ± 0.13	2.0	0.537	0.71	**4.4×10^{-4}**	0.341	6.13 ± 0.14	6.62 ± 0.14	8.0	**0.015**	0.65	**2.2×10^{-7}**	**0.009**
	SE	7.42 ± 0.30	7.12 ± 0.26	−4.0	0.454				5.70 ± 0.19	5.35 ± 0.15	−6.1	0.162			
Pruned biomass*(g/plant/season)	AE	50.89 ± 1.45	48.82 ± 0.91	−4.1	0.271	0.673	**7.4×10^{-9}**	0.346	48.68 ± 2.34	48.78 ± 0.97	0.2	0.970	0.4	**3.5×10^{-6}**	0.378
	SE	28.69 ± 1.13	29.49 ± 2.09	2.8	0.747				32.32 ± 2.69	28.22 ± 2.71	−12.7	0.324			
Harvested fruit yield and marketability															
Plant Yield (kg/plant/season)	AE	2.48 ± 0.06	2.36 ± 0.07	−4.8	0.197	**5.62×10^{-8}**	**1.17×10^{-4}**	**6.08×10^{-5}**	2.17 ± 0.05	2.21 ± 0.04	2.1	0.51	**0.005**	**1.02×10^{-9}**	**2.43×10^{-4}**
	SE	2.37 ± 0.09	1.67 ± 0.08	−29.4	**8.58×10^{-9}**				2.03 ± 0.05	1.70 ± 0.06	−16.7	**2.64×10^{-5}**			
Plant Fruit Number (n/plant/season)	AE	13.26 ± 0.32	11.84 ± 0.29	−10.7	**0.001**	**1.85×10^{-11}**	**3.99×10^{-14}**	**0.005**	13.54 ± 0.35	13.05 ± 0.26	−3.7	0.249	**8.62×10^{-5}**	$<2 \times 10^{-16}$	**0.022**
	SE	11.51 ± 0.40	8.19 ± 0.34	−28.9	**2.25×10^{-9}**				10.65 ± 0.30	8.77 ± 0.28	−17.6	**1.07×10^{-5}**			

(Continued)

TABLE 6 Continued

Parameter	Exp	Red							Orange						
		Control	LBF	Change (%)	LBF *p*-value	LBF	Exp *p*-value	LBF*Exp	Control	LBF	Change (%)	LBF *p*-value	LBF	Exp *p*-value	LBF*Exp
Marketable Fruit Yield (kg/plant/season)	AE	2.17 ± 0.06	2.12 ± 0.06	−2.2	0.552	**4.15×10^{-7}**	**0.004**	**1.08×10^{-5}**	1.88 ± 0.05	2.03 ± 0.04	8.4	**0.016**	0.082	**6.01×10^{-4}**	**3.14×10^{-4}**
	SE	2.28 ± 0.08	1.61 ± 0.07	−29.3	**1.68×10^{-8}**				1.94 ± 0.06	1.60 ± 0.06	−17.5	**5.75×10^{-5}**			
Marketable Fruit Number (n/plant/season)	AE	10.60 ± 0.28	9.98 ± 0.25	−5.8	0.104	**4.71×10^{-6}**	**1.53×10^{-14}**	**0.004**	10.52 ± 0.28	10.96 ± 0.22	4.1	0.223	0.102	**$<2 \times 10^{-16}$**	**0.002**
	SE	8.81 ± 0.46	6.23 ± 0.34	−29.3	**1.33×10^{-5}**				7.81 ± 0.32	6.45 ± 0.31	−17.4	**0.002**			

The values are mean ± standard error of mean (n = 4–40). Change represents (LBF − Control/Control) × 100. For yield and fruit measurements, a season is equal to 248 days which is the length of time between first harvest and last harvest for SE. AE was multiplied by 1.05 to be equivalent to the season length of SE. * is dry mass of pruned biomass. Values in bold text highlight p-values < 0.05.

fraction of light was different across the LBF and control. There was no difference in the amount of blue, green, or red light (PAR) transmitted through the canopy profile between LBF and control, but far-red and NIR light were greatly reduced. Far-red light from 700 nm to 740 nm has been shown to increase photosynthesis (Zhen and Bugbee, 2020), which may lead to lower rates of photosynthesis in the lower canopy in LBF. However, non-photosynthetically active NIR light may result in a hotter canopy in the control than in the LBF, making optimal temperatures in the control during high radiation months harder to maintain.

4.2 Change in biological response under LBF and photoperiod across seasons

While capsicum is known to be a non-photoperiod-sensitive crop in regard to flowering, capsicums have high light requirements, requiring DLIs of 12 mol m^{-2} d^{-1} –30 mol m^{-2} d^{-1} for good productivity, and <12 mol m^{-2} d^{-1} is considered to be light-limited conditions (Cossu et al., 2020; Morgan, 2021). AE was transplanted in autumn under sufficient light conditions, but shortly after planting, the DLI dropped into light-limited conditions, which continued for the first ~4 months of the season with no reduction in DLI due to LBF. The last ~3 months of AE occurred under sufficient light conditions, with a significant reduction in DLI due to LBF. SE was transplanted and established under sufficient DLI, with reductions due to LBF for the first ~3.5 months of growth, then transitioned into light-limited conditions without a reduction in DLI due to LBF for a ~2.5-month period. The final ~1.5 months of the SE season occurred under sufficient light conditions, with a significant reduction due to LBF. Although both AE and SE had similar crop season lengths, ~57% of AE growth occurred under light-limited conditions with no reduction due to LBF, whereas only ~33% of SE growth occurred under light-limited conditions with no reduction due to LBF. LBF DLI levels during SE were also considered light-limited for almost the entire season.

Differences across seasons in light limitation, photoperiod, and reduction in DLI due to LBF at different crop developmental stages impacted crops in different ways. Photoperiod sensitivity is well documented in plant developmental progression, and photoperiod manipulation is used to increase yield by managing supplementary lighting and the timing of planting and harvesting for certain crops (Demers and Gosselin, 1999; Demers & Gosselin, 2002; Garcia and Lopez, 2020). For instance, assimilate partitioning between vegetative and reproductive structures is directly affected by the post-flowering photoperiod in soybean and further influences nodes per plant, which is directly related to seed pod production and thus greatly affects yield (Nico et al., 2019). Plant growth and fruit yield generally increase with photoperiod and DLI maxima, and longer photoperiods with the same DLIs have been shown to increase germination and improve flower growth (Elkins and Iersel, 2020). Extended photoperiods via supplemental lighting have been shown to significantly increase the fruit yield of *C. annuum* (Dorais et al., 1996). However, this increase in production depends on the photoperiod at specific times during the plant developmental cycle, and crops behave differently depending on whether the photoperiod is ascending or descending (Heuvelink, 1995; Dorais et al., 1996; Elkins and Iersel, 2020). In this study, differences in seasonal light across AE and SE generated differences in all biological parameters measured, including the number of buds, flowers developing fruit per plant, growth rate, pruned biomass, and yield.

Overall, the stem growth rate was affected by planting time, with SE exhibiting a faster growth rate than AE; however, LBF did not affect the growth rate for either AE or SE for the red variety or SE for the orange variety. However, LBF generated a marginal increase in the growth rate of the orange variety in the AE. The sufficient light environment during the transplant date in the summer for SE provided an abundant resource for stem elongation, as opposed to the light-limited planting date in the autumn for AE (Tang et al., 2019). A lower overall PAR in LBF could cause LBF to grow faster and taller. However, because LBF's spectral qualities do not increase the R:FR ratio, the plants did not respond with a shade avoidance strategy (etiolation) (Hückstädt et al., 2013).

C. annuum varieties produce buds, flowers, and fruits in a cyclical pattern, and abortion of these organs is common, even when grown in a climate-controlled high-tech glasshouse (Wubs et al., 2009). Different factors influence the presence/abortion of these organs, with the main contributing factors being the light intensity, quality, and photoperiod. LBF increased the presence of buds and flowers in both varieties and developed fruit for the orange variety for AE only. LBF had no impact on the presence of buds, flowers, or fruit for SE, which may be due to the day neutrality of flower induction for this species under sufficient light conditions (Kristiansen and Andersen, 1993). The increase in these components during AE may be due to the slight decrease in the red to blue light ratio by LBF in the absence of canopy-level PAR reduction by LBF in a light-limited environment (Yang et al., 2022). While LBF only had an impact on AE for these traits, there was a significant increase in buds, flowers, and developing fruits for both varieties in SE compared with AE. Plants in the SE grew in higher light periods, with much of the primary production period occurring at a threshold or over sufficient DLI conditions for capsicum. Therefore, plants have adequate resources to produce more buds, flowers, and fruits (Tang et al., 2019; Morgan, 2021).

C. annuum is a non-photoperiod-sensitive species (Yang et al., 2017; Tang et al., 2019); however, there have been mixed findings on leaf area across varied photoperiods. Yamamoto et al. (2008) found no effect on leaf area in response to photoperiod; however, Elkins and Iersel (2020) showed that in response to lower PPFD, plants have been shown to increase leaf size. Although we did not measure leaf area directly, AE had more pruned biomass than SE, suggesting that vegetative growth and leaf area were higher for AE. Plants in AE started developing under light-limited conditions and may have increased leaf area to compensate for low light conditions and reduced photosynthesis per unit leaf area (Rylski and Spigelman, 1986; Rosati et al., 2001; Díaz-Pérez, 2013). Different pruning strategies are employed for different seasonal planting dates to maximize yield (Alsadon et al., 2013; Parniani et al., 2022). For our purposes, pruning regimens were the same across each season, which may have put one season at a yield advantage over the other.

The initial sufficient light environment for SE provided ample light for growth; however, seedling establishment for capsicum was more successful at lower DLI, thus providing a head start on fruit maturation for AE (Yang et al., 2017; Tang et al., 2019). The sufficient light environment for seedling establishment during SE may have unbalanced the plants for reproductive growth, thus producing a lower overall yield compared to AE. In the SE, LBF reduced the DLI season mean (21%) and yield (red: 29%; orange: 16%), which is supported by Marcelis et al. (2006), who found that every 1% reduction in DLI resulted in a ~1% reduction in yield. However, the yield was not affected by LBF in AE, even though the DLI season mean was reduced (18%). The yield was marginally higher for the orange variety in AE (2.1%); however, this marginal increase in yield can be considered no change. The yield reduction in SE seems to be related to light-limited thresholds for capsicum. For instance, during AE, DLI reduction by LBF started when both control and LBF were within sufficient light conditions, while during SE, in periods when LBF reduced DLI, LBF was either at threshold or in light-limited conditions, while the control was under sufficient light conditions. This differential impact of LBF across AE and SE is likely due to the combination of the (1) Initial light environmental conditions during seedling establishment, (2) amount of time each crop spent with significant light reduction due to LBF, and (3) the amount of time that the control was under sufficient light conditions, while the LBF was at or below the threshold.

5 Conclusion

Sensor technology is an important component of PC agriculture for improving crop growth and maximizing yield. The current study demonstrates the use of light sensors (e.g., net radiometer, diffuse light sensor, spectro-radiometer, and PAR sensors) to understand the energy balance, changes in light quantity, and light quality. We assessed the impact of a residential building film (LBF) on light quantity and quality, and subsequently on the growth and yield response of *C. annuum* L. LBF reduced light quantity and altered light quality. These changes in light environment had differential impacts on crop development and yield of *C. annuum* depending on the planting time, the amount of time the crop grew during light-limited versus sufficient light conditions, and whether LBF reduced DLI.

We conclude that (1) the sensors used in the current study were able to characterize the light quantity, light quality, and light energy balance that contribute to heat generation in glasshouses; (2) LBF reduces total net radiation but increases LW radiation which may contribute to heat load and negatively impact cooling capacity; (3) LBF was not appropriate for year-round capsicum production because it reduced yield in SE despite potential reductions in energy use; (4) LBF may be useful for producing crops during the high radiation months of the year; and (5) for future assessment of LBF and agricultural cover materials aimed at reducing energy usage, the additional measurement of both roof and crop canopy temperature should be used to further inform the energy balance within a PC growing environment.

Data availability statement

The original contributions presented in the study are included in the article/supplementary files, further inquiries can be directed to the corresponding author.

Author contributions

CM: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft. SC: Conceptualization, Methodology, Supervision, Writing – review & editing. NK: Project administration, Writing – review & editing. WL: Project administration, Writing – review & editing. CC: Conceptualization, Funding acquisition, Writing – review & editing. OG: Funding acquisition, Writing – review & editing. Z-HC: Funding acquisition, Supervision, Writing – review & editing. DT: Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

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

Publisher's note

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EDITED BY
Giao Nguyen,
Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia

REVIEWED BY
Yaiza Gara Padilla,
Valencian Institute for Agricultural Research (IVIA), Spain
Kehinde Erinle,
Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia

*CORRESPONDENCE
Vijay Joshi
✉ Vijay.Joshi@tamu.edu

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Biochemical repercussions of light spectra on nitrogen metabolism in spinach (*Spinacia oleracea*) under a controlled environment

Moazzameh Ramezani[1], Dalton Thompson[1], Matte Moreno[1] and Vijay Joshi[1,2*]

[1]Texas A&M AgriLife Research and Extension Center, Uvalde, TX, United States, [2]Department of Horticultural Sciences, Texas A&M University, College Station, TX, United States

Introduction: Selecting appropriate light spectra of light-emitting diodes (LEDs) and optimal nutrient composition fertilizers has become integral to commercial controlled environment agriculture (CEA) platforms.

Methods: This study explored the impact of three LED light regimes (BR: Blue17%, Green 4%, Red 63%, Far-Red 13% and infrared 3%, BGR; Blue 20%, Green 23%, Red 47%, Far-Red 8% and infrared 2%; and GR; Blue 25%, Green 41%, Red 32%, and Far-Red 2%) and nitrogen levels (3.6 and 14.3 mM N) on spinach (*Spinacea oleracea*).

Results: Under limited nitrogen (3.6 mM), BGR light increased the fresh shoot (32%) and root (39%) biomass than BR, suggesting additional green light's impact on assimilating photosynthates under suboptimal nitrogen availability. Reduced chlorophyll (a and b) and carotenoid accumulation, electron transport rate (ETR), and higher oxalates under limited nitrogen availability highlighted the adverse effects of red light (BR) on spinach productivity. Increased activities of nitrogen-associated enzymes (GOGAT; Glutamate synthase, GDH; NADH-Glutamate dehydrogenase, NR; Nitrate reductase, and GS; Glutamine synthetase) in spinach plants under BGR light further validated the significance of green light in nitrogen assimilation. Amino acid distributions remained unchanged across the light spectra, although limited nitrogen availability significantly decreased the percent distribution of glutamine and aspartic acid.

Conclusion: Overall, this study demonstrated the favorable impacts of additional green light on spinach productivity, as demonstrated under BGR, than GR alone in response to nitrogen perturbation. However, the exact mechanisms underlying these impacts still need to be unveiled. Nevertheless, these outcomes provided new insights into our understanding of light spectra on spinach nitrogen metabolism.

KEYWORDS

spinach, light spectrum, nitrogen, amino acids, minerals

1 Introduction

Since the onset of the coronavirus pandemic, the adoption rate of Controlled Environmental Agriculture (CEA) systems worldwide has been rising (Van Delden et al., 2021; Cowan et al., 2022; Zhang et al., 2022). Even though CEA-produced vegetables frequently carry price premiums, their share in fresh vegetable production will continue to expand due to increasing consumer demand for local food options. As an indoor food production system, CEA has offered a technological breakthrough to solve several problems associated with traditional farming and mitigate environmental and social challenges at the food-energy-water nexus (Lubna et al., 2022; Vatistas et al., 2022; Dsouza et al., 2023). Even if the CEA industry is booming, the science-based interventions to optimize the technical precision that identifies suitable light spectrums and nutrient management strategies to recover desired nutritional benefits need continual attention (Gómez et al., 2019; Sharathkumar et al., 2020; Neo et al., 2022).

Spinach is popular among vegetables due to its nutritional benefits, contributing to an estimated 40% of the leafy greens market (Batziakas et al., 2019; NASS, 2021). Although spinach accumulates many nutritional components, its accumulation is subjective to the growing environment. Maximizing the indoor production of spinach without compromising its nutrients is critical to its nutritional quality. Nitrogen (N) is an expensive input critical to maximizing plant productivity. Like most leafy greens, spinach requires excessive amounts of N fertilizers to produce higher biomass regardless of the production system (Markovic et al., 1988; Elia et al., 1998; Canali et al., 2014; Abdelraouf, 2016; Frerichs et al., 2022). Enhancing crop nitrogen use efficiency (NUE) without compromising quality and yield has become an apparent crop production strategy. Most higher plants reduce nitrate, the inorganic form of N, into an organic form, such as ammonia, by distinct enzymatic reactions initiated by nitrate reductase (NR) (Crawford, 1995). Ammonia is assimilated into amino acids glutamine and glutamate via individual isoenzymes of glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH) (Ireland and Lea, 1999). Spinach's nitrogen use efficiency (NUE) is poor due to the shallow root system (Stagnari et al., 2007; Marvi, 2009) and nitrate reduction efficiency (Neeteson and Carton, 2001; Koh et al., 2012). Although enhancing the NUE in spinach by exploiting existing genetic diversity has been proposed (Chan-Navarrete et al., 2014; Chan-Navarrete et al., 2016), N uptake is subjective to the production system. In production systems, where the supply of N or other nutrients is not limited, the uptake and assimilation of micronutrients or other phytochemicals are primarily subjected to manipulating environmental parameters.

Environmental optimization is crucial to improving the profitability of CEA platforms. As the CEA industry proliferates, manipulating the growing environment to maximize nutrient recovery needs continual research. Selecting appropriate light spectra of light-emitting diodes (LEDs) has become integral to commercial CEA platforms due to their flexibility in tailoring the light spectrum to maximize production (Balázs et al., 2022; Sheibani et al., 2023). Manipulating spectral quality has significantly impacted nutritional qualities among a wide range of high-value specialty crops (Hasan et al., 2017; Jones, 2018; Paradiso and Proietti, 2022). The different quantum efficiency and photoelectric conversion efficiencies of red and blue LED lights or their combinations result in varied energy consumption. Red and blue wavelengths impact photosynthetic performance(Johkan et al., 2010), morphogenesis (Dou et al., 2020; Chen et al., 2021), and metabolic composition (Kopsell et al., 2015; Trivellini et al., 2023) due to maximal absorption by chlorophyll a and b. However, the misconception of these spectra being the most efficient is challenged by several studies showing the positive impacts of green light on photosynthesis (Terashima et al., 2009; Hogewoning et al., 2012; Smith et al., 2017). As per the Emerson effect (Emerson et al., 1957), simultaneous exposure to red and far-red LED illumination enhances photosynthesis in plants, mainly morphological features such as leaf length (Li and Kubota, 2009) and yield or biomass (Kim et al., 2020). The higher absorbance of red and blue lights yields a higher quantum yield of CO_2 assimilation (Q_Y, moles of CO_2 assimilated per mole of photons) than green light (Liu and Van Iersel, 2021).

On the other hand, despite its lower absorptance, green light can penetrate and excite chlorophyll deeper in leaves. Several studies have shown the significance of far-red light (700–800 nm) in mediating plant growth and developmental processes (Islam et al., 2014; Demotes-Mainard et al., 2016; Park and Runkle, 2017; Zhen and Bugbee, 2020). The interaction of these spectra on productivity is not always synergistic (Liu and Van Iersel, 2021) and is defined by the plant species, developmental stages, and growing conditions. Nevertheless, the impact of N or light spectrum on the productivity or N assimilation in spinach under indoor systems remains to be tested.

In the present study, we have evaluated the interaction of LED lights differing in the composition of ratios between blue, green, and red-light spectra under two N regimes to understand its impact on spinach performance in a soil-less media under a controlled growth chamber. Although each production system has unique challenges, using a soilless matrix facilitates uniform growth and control of nutrient media due to its inert chemical composition, allowing productivity assessment as a function of N or light applied. We have shown that supervised machine learning effectively predicts the root traits in a uniform soil-less matrix (Awika et al., 2021). The results of this work should serve as a reference for additional light optimization in commercial indoor spinach production and improve our understanding of the effect of light quality on N assimilation and biochemical attributes in spinach.

2 Materials and methods

2.1 Growth conditions

The experiment was performed in a controlled growth chamber at the Texas A&M AgriLife Research and Extension Center, Uvalde, Texas. The spinach variety 'Space' seeds were planted in a growth medium in pots (10.2 cm x 10.2 cm x 8.9 cm) containing Turface (Turface Athletics^{TM} MVP, PROFILE Products LLC, Buffalo Grove, Illinois, USA).

Plants were grown under three light-emitting diodes (LED) lights (Supplementary Figure 1) (A) BR light (Red Bloom spectrum; Active Grow, Seattle, WA), which uses 17% Blue, 4% Green, 63% Red, 13% Far-Red and 3% infrared, (B) BGR light (Red Bloom Pro Spectrum, Active Grow, Seattle, WA) formulated with 20% Blue, 23% Green, 47% Red, 8% Far-Red and 2% infrared; and (C) GR light (White Pro Spectrum, Active Grow, Seattle, WA) formulated with 25% Blue, 41% Green, 32% Red, and 2% Far-Red spectra at the light intensity of 200 µmol m^{-2} s^{-1} biologically active radiation (400–800 nm) inside a growth chamber maintained under a 12/12 h light/dark cycle, 22°C, and 75% relative humidity.

After the seedling emergence, plants were fertilized with Peters® professional ready mix (5-11-26, Everris NA Inc., Ohio, USA) every four days. Two concentrations of nitrogen - LN (3.6 mM) and HN (14.3 mM) were used for low and high N management. An additional N for the high N was provided using calcium nitrate, and equivalent calcium was compensated for the low N, as detailed earlier (Joshi et al., 2020; Awika et al., 2021).

2.2 Determination of biomass, minerals, and NUE

Fresh weight (mg) of root and leaves was measured at harvest (55 days after germination) using an analytical balance. The dry weight was measured by oven-drying at 70°C for 72 h. The plant samples were analyzed for total N (TKN), NO_3^{-} and NH_4^{+} using an EasyChem Plus analyzer (Chinchilla Scientific, Oak Brook, IL, USA). Total elemental analysis was conducted using ICP-OES (Thermo Scientific™ iCAP™ 7000 Plus Series, Waltham, MA, USA). The nitrogen use efficiency (NUE) percentage was calculated as a ratio of TKN x dry biomass (g)/N input (g) separately under HN and LN. For the rest of the minerals (Ca, Mg, K, P, Fe, Cu, Zn), nutrient utilization efficiencies (NuUtE) were calculated by taking the ratio of dry biomass per plant to mineral content using established methods (Corrado et al., 2021). Individual mineral use efficiencies were obtained by taking the ratio of individual mineral amounts recovered from plant tissue to the concentration (ppm) applied through fertilizers.

2.3 Amino acid extraction and quantification with UPLC-ESI-MS/MS

Approximately 10 mg lyophilized plant tissue samples were homogenized into a fine powder in a Harbil model 5G-HD paint shaker (Harbil, Wheeling, IL, USA) using 3 mm Demag stainless steel balls (Abbott Ball Company, CT, USA). Total free amino acids were extracted by suspending the homogenized samples in 100µL of 20mM cold HCl per mg of tissues, incubating on ice for around 20 minutes, and then centrifuging at a speed of 14,600xg for 20 min at 4°C. The extracts were filtered through a 96-well 0.45-µm-pore filter plate (Pall Life Sciences, USA). The filtrates were used for derivatization using AccQ•Tag3X Ultra-Fluor™ kit (Waters Corporation, Milford, MA, USA) as per the manufacturer's protocol. L-Norvaline (TCI AMERICA, USA) was used as an internal control. Calibration curves were built using TargetLynxTM Application Manager (Waters Corporation, Milford, MA, USA). UPLC-ESI-MS/MS analysis was performed using Water's Acquity H-class UPLC system equipped with Waters Xevo TQ mass spectrometer and electrospray ionization (ESI) probe. Water's MassLynx™ software was used for instrument monitoring and data acquisition. The data integration and quantitation were conducted using Waters TargetLynx™ software.

2.4 Measurement of chlorophyll and carotenoid

The chlorophyll content (µmol of chlorophyll per m² of leaf surface) was measured using a portable chlorophyll content meter (MC-100, Apogee Instruments, Inc., Logan, UT, USA) from the fully expanded leaves of 6-week-old plants. Freeze-dried spinach tissue powders (10mg) were vortexed with 80% (v/v) acetone, sonicated (5 min, room temperature), and centrifuged (14,600×g, 5 min). The supernatant was used to measure absorbance at 470 nm, 645 nm, and 663 nm using a Multiskan GO microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Chlorophyll and carotenoids were determined using preestablished equations (Lichtenthaler and Wellbur, 1983). Chlorophyll fluorescence parameters were recorded using the portable fluorometer FluorPen 110 (Photon Systems Instruments, Czech Republic) after dark, adapting the leaves for 30 min with the leaf clips on fully expanded leaves of each plant. The F_o values represent the chlorophyll fluorescence emission associated with energy losses in the light-harvesting complexes of PSII (Kalaji et al., 2017), and F_m values show the maximum level of fluorescence from the dark-adapted leaves when all PSII reaction centers are "closed" with a saturating flash of light were measured from fully expanded spinach leaves and used to derive ratios (F_v/F_m; F_m/F_0; F_v/F_0). FT (instantaneous chlorophyll fluorescence) and Q_Y (quantum yield) were instant measurements. The polyphasic chlorophyll fluorescence (OJIP) transients were measured on fully developed leaves following a 20-minute dark adaptation. The PSII parameters obtained from the OJIP transient ($F_o = F_{30\mu s}$, minimum fluorescence intensity; $F_j = F_{2ms}$, fluorescence intensity at the Jstep; $F_i = F_{30ms}$, fluorescence intensity at the I-step; F_p = maximum fluorescence intensity at the peak P of OJIP) were analyzed using Strasser method (Strasser et al., 2010). Stomatal conductance (gsw), photosynthetic electron transport rate (ETR), and PSII actual photochemical quantum yield (PhiPS2) were measured on a fully expanded leaf using LI-600 Porometer/Fluorometer (LI-COR Biosciences, Lincoln, NE, United States).

2.5 Enzyme activity and oxalate assays

The nitrate reductase (NR) activity was measured using the established method (Yaneva et al., 2002). Spinach tissue was homogenized in 100 mM phosphate buffer (pH 7.5) and then centrifuged at 14,600 x g for 15 min at 4°C. 100 uL supernatant extract was added to 200 µmol KNO_3 and 0.2 µmol nicotinamide adenine dinucleotide, to estimate the NR activity. The reaction was stopped by adding 50 μL 1 m zinc acetate after 20 min incubation at 30°C. The mixture was centrifuged at 7600 x g for 5 min, and the absorbance was recorded at 540 nm using a MultiSkan Go microplate reader (Thermo Scientific, Waltham, MA, USA). One unit of NR activity was defined as the nitrite nitrogen produced content per gram of fresh weight per hour ($\mu g\ g^{-1}\ h^{-1}$).

Glutamine Synthetase (GS) and Glutamate Synthase (GOGAT) activities were determined by extracting spinach tissue samples with 50 mM phosphate buffer (2 mM EDTA, 2 mM dithiothreitol, 1% insoluble polyvinylpyrrolidone, and 1.5% soluble casein, pH = 7.5) and centrifuged for 30 min at 12,600 x g. The GS and GOGAT activities were measured as per the established protocols (Cánovas et al., 1991). One unit activity of GS was expressed as mmol g-glutamylhydroxamate formed per gram per minute and GOGAT activity as mmol NADH oxidized per gram per minute.

NADH-Glutamate dehydrogenase (GDH) enzyme activity was measured using an established protocol (Robinson et al., 1991). One unit of GDH activity was expressed as oxidization or reduction of 1 μmol NADH per min.

The oxalate assay kit (Colorimetric) (ab196990) was used following the manufacturer's instructions (Abcam, Cambridge, MA, USA) to detect oxalate levels in spinach leaves. The plant extracts were prepared by homogenizing 15 mg tissue, followed by incubation with assay buffer and centrifugation at 10,000 x g for 5 min. The reaction mixture was added and incubated for 30 min, and the optical density at 450 nm was measured using a MultiSkan Go microplate reader (Thermo Scientific, Waltham, MA, USA). The concentration of oxalate was then calculated from a standard curve.

2.6 Statistical analysis

The data presented corresponds to the mean value ± standard error. Descriptive and summary statistics, analysis of variance (ANOVA), and the principal component analysis (PCA) for various measurements were calculated using JMP 14.0.0 (SAS Institute, Cary, NC, USA). One-way and two-way ANOVA was also conducted where applicable, with $\alpha = 0.05$ and significance set at $p < 0.05$. The significant differences among treatment groups were determined using the Turkey Kramer HSD at $p = 0.05$, and letter groupings were generated using a 5% significance level. The normality of the distribution was tested by a P-value < 0.05 in a Shapiro–Wilk test. The data was subjected to PCA and biplots to visualize general clustering, trends, and differences among samples for free amino acids and mineral contents.

3 Results

3.1 Effects of the light spectrum and nitrogen on biomass and NUE

We validated the impact of the nitrogen levels on the performance of spinach under three light spectrums by comparing the fresh and dry biomass and NUE using two N regimes. The Analysis of Variance (ANOVA) confirmed significant direct effects due to N for shoot and root biomass and the interaction effects due to N and light spectra only for dry root biomass (Supplementary Table 1). The shoot fresh biomass under three light spectra did not differ significantly under high N, although BGR-exposed plants had higher biomass at low N availability (Figure 1). The percentage increases in the fresh leaf biomass due to high N were comparable among BR (66%) and GR (61%) lights but were lower in the presence of BGR (41%) light (Supplementary Table 2). The NUE $(g/g)^{-1}$ values based on shoot dry biomass across lights were comparable within the nitrogen treatment but were much higher in magnitude when the N was suboptimal (Supplementary Table 2). The analysis of root fresh weight revealed that BGR-exposed root biomass was higher (~35%) than other lights under limited N availability, with no significant

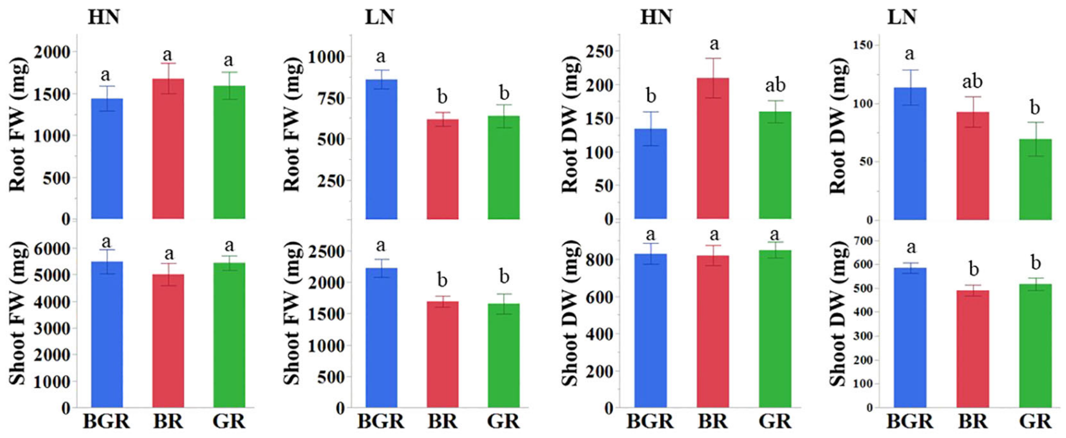

FIGURE 1
Effects of light treatments (BGR, BR, GR) on fresh (FW) and dry (DW) shoot and root biomass under high and low nitrogen. Different letters indicate statistically significant differences (P ≤ 0.05); N=10 plants.

differences among the light treatments under high N treatment. The root dry biomass under BR light was higher than BGR when N was not limiting. On the other hand, BGR-exposed plants under low nitrogen had higher root dry biomass than GR.

3.2 Interaction effects of nitrogen and light spectrum on chlorophyll accumulation and carotenoid content in spinach

Light spectra or their interaction with N significantly impacted the Chlorophyll a and carotenoid contents (Supplementary Table 1). High nitrogen enhanced Chl a, b, and carotenoid accumulation across lights (Figure 2) relative to low N. The Chl a and b content in BGR and GR light spectra under limited N was significantly higher (~46% and ~39%) than in BR lights. Similarly, the BR light decreased carotenoid accumulation by 27% under low N availability compared to BGR and GR. The chlorophyll (µmol of chlorophyll per m² of leaf surface) concentrations across lights showed significant changes in response to N and varying light spectra, but their interaction was insignificant (Figure 3; Supplementary Table 1). BR light plants accumulated significantly lower chlorophyll (11% under high N and 17% under low N) than the other two light spectra.

3.3 Chlorophyll fluorescence measurements

Chlorophyll fluorescence was recorded on fully expanded leaves of each plant using a FluorPen FP 110 (Supplementary Figure 2). F_0 in the dark-adapted spinach leaves was lower in BGR and GR lights than in BR. No change in F_0 readings was seen in response to the N status for all light treatments. Excepting BR light under both N regimes, F_m (maximum fluorescence) values were unchanged for BGR and GR lights. The F_v/F_m and F_v/F_0 ratios were calculated to assess the efficiency of photochemical activities in PSII. The F_v/F_m and F_v/F_o did not differ significantly due to N treatments across lights. The effective quantum yield of photosystem (PS) II photochemistry (Q_Y and F_t; instantaneous chlorophyll fluorescence) and OJIP analysis are often used as suitable markers for determining plant response to various stressors. Q_y or F_t values did not differ significantly across light spectra. When subjected to limited nitrogen availability, the plants exhibited higher F_t and lower Q_Y values under GR (Supplementary Figure 3).

A significant interaction between light spectra and N was observed for the electron transport rate (ETR) and stomatal conductance (gsw) ($p < 0.07$) in spinach plants (Figure 4; Supplementary Table 1). Unlike unchanged conductance under BR light, a decrease in the stomatal conductance was recorded under BGR and GR with declining N content. In contrast, regardless of the N status, the electron transport rate (ETR, µmol m^{-2} s^{-1}) remained the same under all lights, although it was significantly lower under BR. The quantum efficiency of PSII (PhiPS2) reduced with N availability but did not respond to spectral changes.

The non-destructive polyphasic OJIP chlorophyll fluorescence transients' analysis under different light spectra was used to evaluate the photosynthetic function in the dark-adapted leaves. In all light spectra, plants growing under low N showed lower induction in Chl fluorescence intensity during all steps of the OJIP graph (F_0, F_J, F_I, and F_m) than high N. In contrast, Chl fluorescence intensity was consistently high in BR light during OJIP transitions (Figure 5).

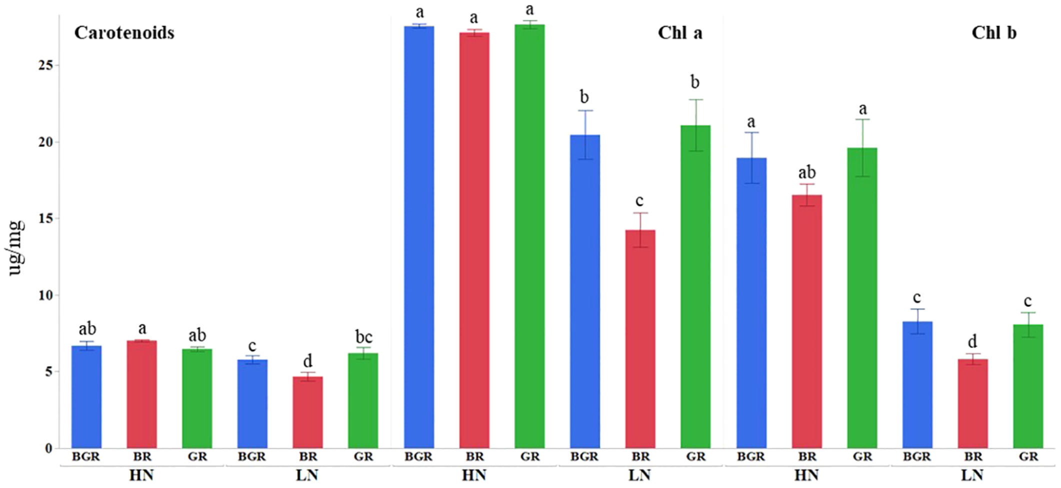

FIGURE 2
Effects of light treatments (BGR, BR, GR) and nitrogen levels (HN, LN) on chlorophyll (Chl a and Chl b) and carotenoid accumulation in spinach. Different letters indicate statistically significant differences ($P \leq 0.05$); N=5.

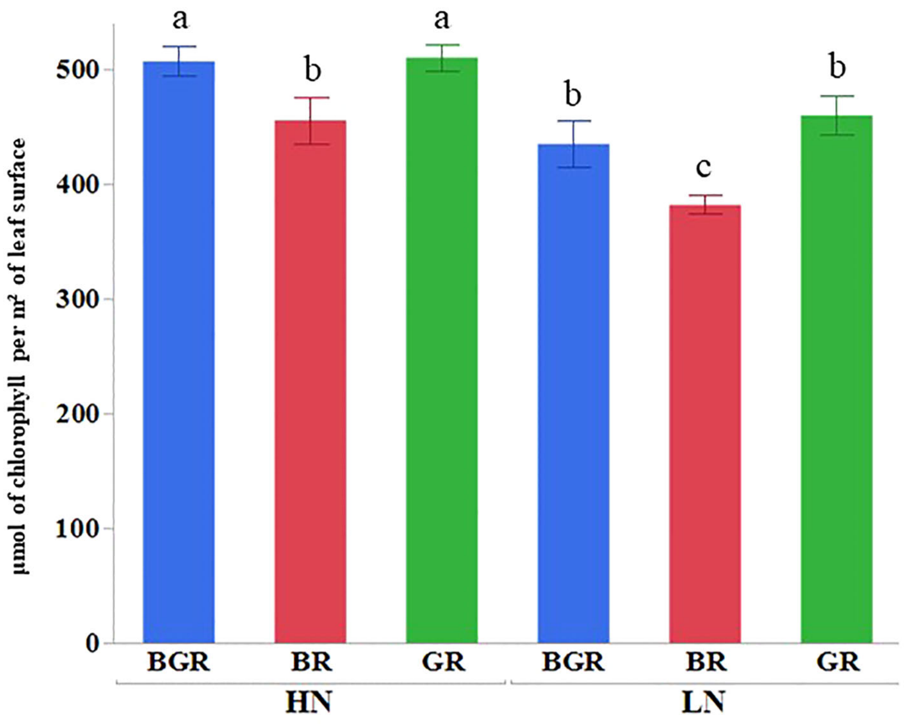

FIGURE 3
Chlorophyll (mol of chlorophyll per m^2 of leaf surface) content in spinach leaves under different lights (BGR, GR, BR) and nitrogen levels (HN, LN). Different letters indicate statistically significant differences (P ≤ 0.05); N=4.

3.4 Effects of light and N on activities of enzymes associated with nitrogen assimilation and oxalate contents

The analysis of variance (ANOVA) confirmed significant primary light x enzyme activities and nitrogen x enzyme activities interaction effects for all the enzymes (NR; Nitrate reductase, GDH; Glutamate dehydrogenase, GS; Glutamine synthase, GOGAT; Glutamate synthase) in the spinach leaves (Supplementary Table 3).

The GDH activity under BGR light was significantly higher under high N than BR and GR (Figure 6). GDH activity under limited N was significantly inhibited under BR light compared to BGR and GR. The GOGAT activity in spinach leaf was ~ 40% higher under the BGR light than under the GR and BR lights when N was not limiting. Unlike the BR light that decreased GOGAT under LN, the GOGAT activities under BGR and GR lights did not respond to N changes. GS activity significantly dropped under BR and GR relative to BGR lights when N was limiting. The NR activities showed no differences in response to N levels under BR or GR lights. The NR activity under BGR light was significantly higher than BR (104%) and GR (76%) when N was surplus.

Oxalate content in spinach leaves did not differ significantly under different spectra when available N was excess. However,

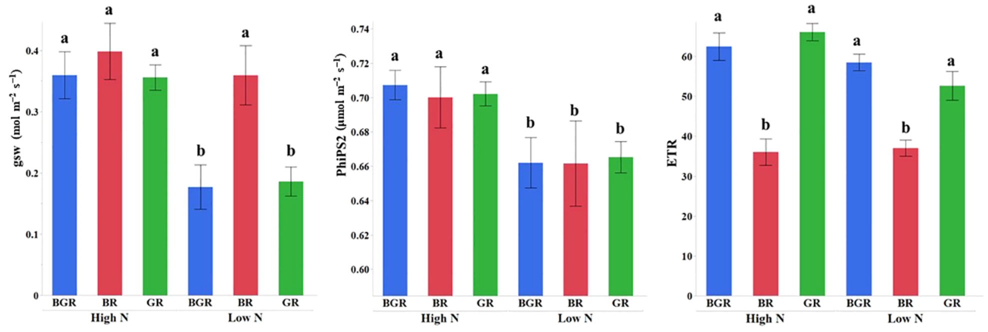

FIGURE 4
Effect of the light spectrum treatments (BGR, GR, BR) and nitrogen levels (HN, LN) on stomatal conductance (gsw, mol m^{-2} s^{-1}), electron transport rate (ETR, µmol m^{-2} s^{-1}), the quantum efficiency of PSII (PhiPS2). Different letters indicate statistically significant differences (P ≤0.05); N=4.

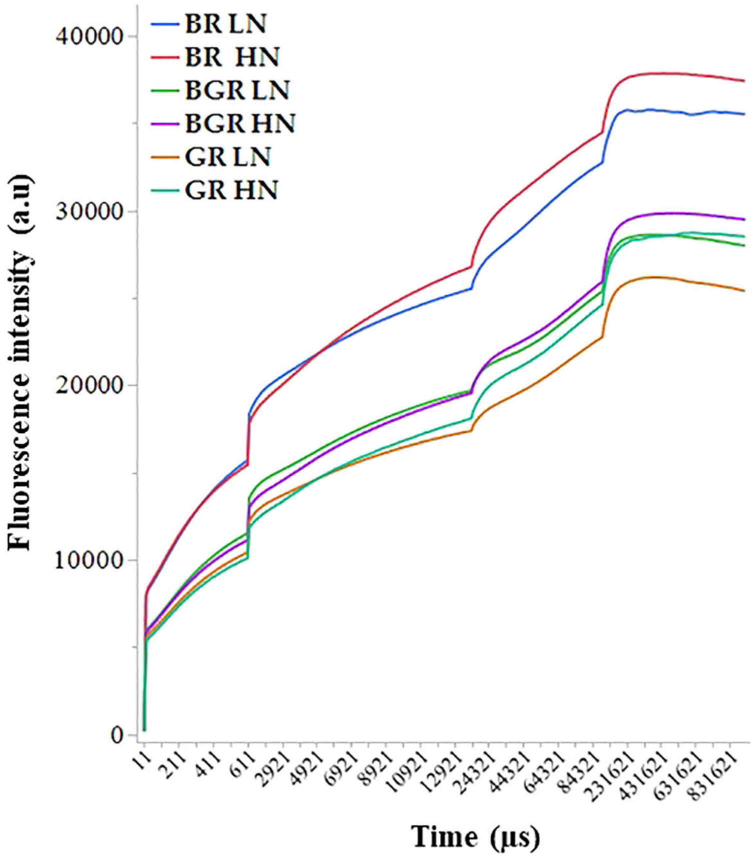

FIGURE 5
The intensity of Chlorophyll fluorescence during different steps of the OJIP curve exhibited by spinach leaves grown under varied light spectra (BGR, BR, GR) and nitrogen (HN and LN). Data was collected from four independent plants.

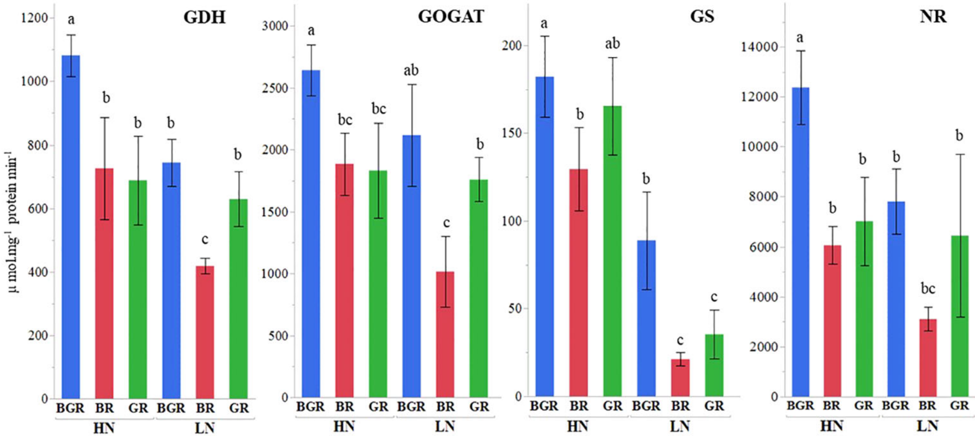

FIGURE 6
Effects of light spectra (BGR, BR, GR) and nitrogen levels (HN, LN) on nitrogen-associated enzymes GOGAT (Glutamate synthase), NR (Nitrate Reductase), GDH (Glutamate dehydrogenase), GS (Glutamine synthase), in the spinach leaf. Different letters indicate statistically significant differences ($P \leq 0.1$). Each bar represents the mean of three replicates, and the error bars represent ± SE.

unlike BGR or GR lights, no significant change in the oxalate content was seen under BR light when N was limiting (Figure 7).

3.5 Accumulation of free amino acids in response to N and light spectra in spinach

Free amino acid accumulation and partitioning between leaf and root tissues across light treatments were evaluated in response to varied N levels. The analysis of variance (ANOVA) indicated that the percent distribution of most free amino acids showed significant differences between tissue type (leaf vs. root), light spectra, and N treatments (Supplementary Table 4) at p<0.1. Most amino acids differ significantly in response to N availability, tissue types, and their interaction. The three-way interaction between light, N, and tissue type was significant for asparagine, histidine, and alanine. Interaction between light and nitrogen was highly significant for less abundant amino acids (methionine, threonine) but also showed trends for N-rich abundant amino acids like asparagine and glutamine. Light alone significantly impacted histidine, phenylalanine, and alanine. Among the most abundant amino acids, glutamine and aspartic acid accumulation were enhanced under high N, irrespective of light spectra (Figure 8) in shoots. Under sufficient N availability, BR light accumulated significantly higher glutamine but lower serine and alanine in the shoot tissue. The percent distribution of glutamine, GABA, asparagine, aspartic acid, and alanine were higher in root tissue (Supplementary Figure 4). The percent distribution of the most abundant glutamine was significantly higher in the roots of BGR and GR-exposed plants than in BR under both N rates. The percent GABA distribution in the roots of plants under low N was significantly higher under high N in all respective light spectra.

To obtain a global overview of the effects of light spectra and nitrogen on the measured free amino acid pool sizes, we subjected the relative changes to Principal Component Analysis (PCA) (Figure 9). PCA projection demonstrated that the maximum variability in the data set differentiated between low and high nitrogen, with the first component (PC1) covering 54% of the data variance in shoot tissue. Both the principal components failed to discriminate between light spectra, suggesting their limited impact on the amino acid metabolome. Most N-rich amino acids, such as arginine, asparagine, glutamine, aspartic acid, and glutamic acid, were positively associated with HN in shoot tissue. While besides serine, GABA, and alanine, other amino acids were associated with LN in the PC1. The analysis of root data revealed that the largest source of variability in the dataset distinctly differentiated between nitrogen levels and light treatments. The first component (PC1) accounted for 52% of the variance in root tissue, emphasizing differentiation between low nitrogen and GR and BGR spectra while high nitrogen and BR spectra. Results showed an association between BR light and glutamic acid, serine, phenylalanine, and aspartic acid. On the other hand, root glutamine was associated with BGR and GR lights under limited N.

3.6 Effects of light and nitrogen on mineral accumulation and their Interactions

The direct impact of the light spectrum and N availability on mineral absorption is not documented in spinach. Principal

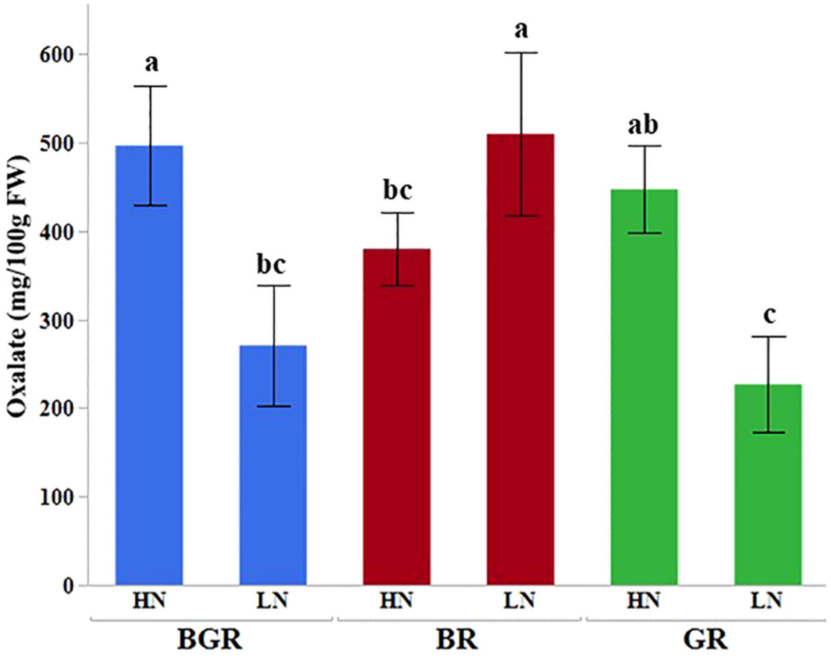

FIGURE 7
Effects of light (BGR, BR, GR) and nitrogen levels (HN, LN) on the oxalate content in the spinach. Each bar represents the mean of three replicates, and the error bars represent ± SE. Different letters indicate significant differences (P ≤0.05).

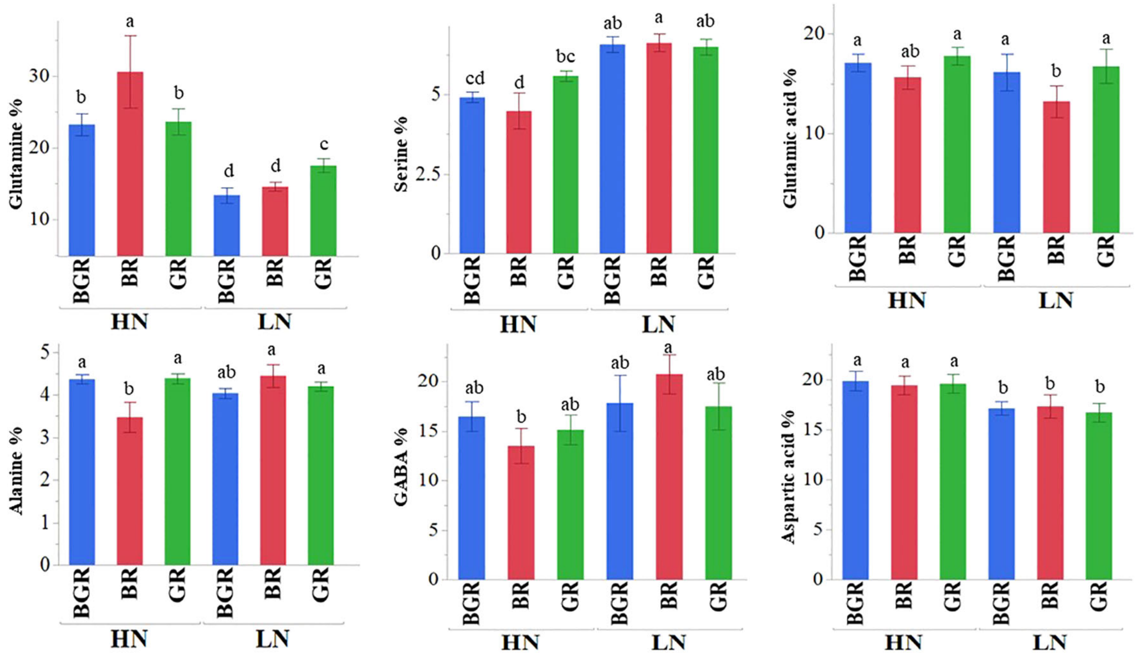

FIGURE 8
Changes in the percent accumulation of selective abundant free amino acids in spinach in leaf tissues under BGR, GR, BR lights, and nitrogen levels (HN, LN). Each bar represents the mean of six replicates ± SE. Different letters indicate significant differences (P ≤0.05).

Component Analysis (PCA) was utilized to comprehensively overview the impacts of light spectra and nitrogen on the mineral accumulation and the relative changes (Figure 10). The Biplot graph PCA projection revealed that the most significant variations in the dataset distinctly distinguished between nitrogen levels and light. The first principal component (PC1) accounted for 32% of the variance in leaf tissue, highlighting the differentiation between nitrogen treatments. The high N was positively associated with TKN, NO_3, HN_4-N, K, and Cu.

In contrast, Ca, Se, Fe, and Cr were tightly associated with each other but negatively correlated with N-rich compounds. The light spectra have limited influence on the accumulation of macro or micronutrients. Most macro/micronutrient accumulation did not alter significantly across light spectra but was significantly higher for Total N (TKN nitrates (NO_3) and magnesium in GR under limited N (Supplementary Figure 5).

4 Discussion

We investigated biochemical alterations in the nitrogen metabolism of spinach plants grown in varied light quality using

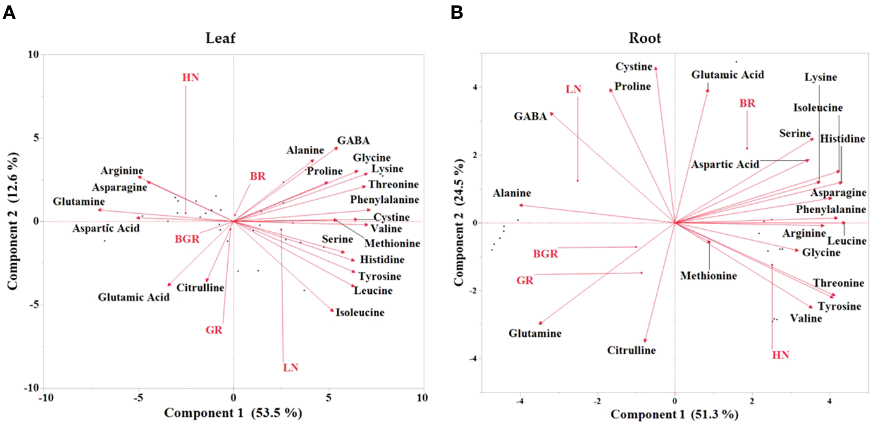

FIGURE 9
Principal component analysis (PCA) of free amino acids in spinach plant under different lights and nitrogen treatments in leaf **(A)** and root **(B)** tissues. Bi-plot for the first two principal components (PC) for free amino acids (scores) and treatments (loadings) as vectors for BGR, GR, BR lights and HN (High nitrogen) and LN (Low nitrogen).

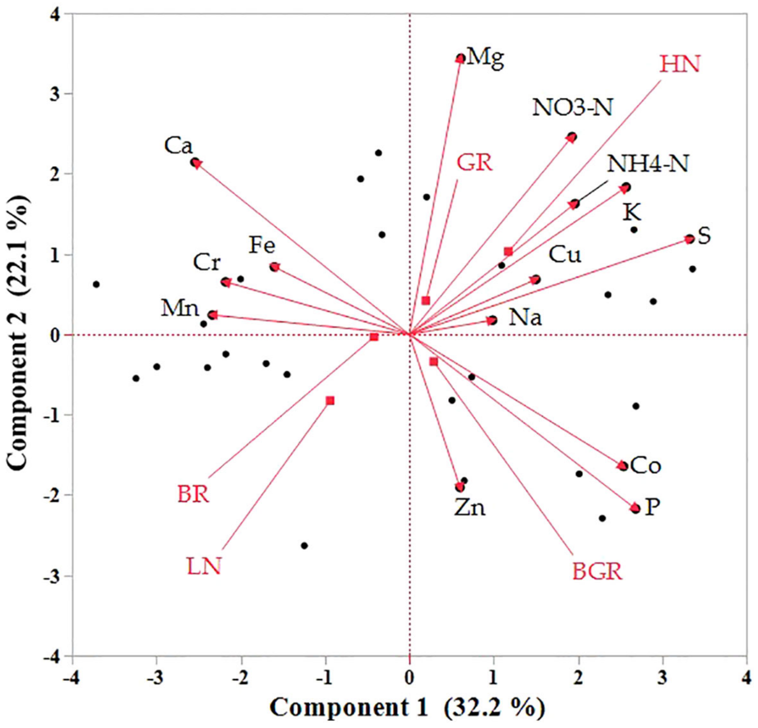

FIGURE 10
Principal component analysis (PCA) demonstrates the clustering of macro and microelements in response to N and light spectra into the first two principal components in the shoot. Bi-plot for the first two components (PC) for minerals(scores) and treatments (loadings) as vectors for BGR, BR, and GR lights and N levels (HN, LN).

blue and red light-emitting diodes (LEDs) along with a green light source. Due to their improved efficiency, LEDs are extensively used as supplemental lighting for indoor production of leafy greens and other horticultural crops. Specific narrow-banded light spectra within the electromagnetic spectrum affect the biochemistry and structure of photosystems, consequently influencing the nutritional content of the plants. Many physiological and molecular changes induced by specific light spectra and their interaction with nitrogen availability are poorly understood. Furthermore, with CEA poised to be prominent in commercial vegetable production, precise optimization of light quality to harness most nutraceutical benefits of leafy greens like spinach needs continual research.

Red and blue LED light or their combinations are popularly used for CEA production systems as leaves exhibit high absorbance and low reflectance in these spectral bands, promoting plant growth and development (Naznin et al., 2019). As the absorption of photons by chloroplasts near the adaxial surface induces heat dissipation of excess excitation energy with only little available to the ones in deeper leaf tissue (Sun et al., 1998; Nishio, 2000), it is suggested that blue and red photons are used less efficiently than green photons (Liu and Van Iersel, 2021). Several studies have underlined the importance of green light for uniform photosynthesis (Sun et al., 1998; Terashima et al., 2009; Hogewoning et al., 2012; Smith et al., 2017). In our study, we compared spectral composition that dominantly peaked for red light (red 63%, far-red; 13%, infrared; 3%) to that of two spectral distributions that prominently involved green light along with varied compositions of red and blue lights (Supplementary Figure 1). It has been argued that green light penetrates deeper into plant tissue than other colors to excite photosystems in deeper cell layers. In particular, measurements of chlorophyll fluorescence and other optical parameters within spinach leaves showed that light was absorbed in greater depths, with 50% of blue and green light reaching 125 and 240μm deep, respectively (Vogelmann and Evans, 2002) and blue light absorption by the lowermost chloroplasts was <5% of that of the uppermost (Evans and Vogelmann, 2003). Our results demonstrated that compared to BR (4% green light), incremental increases in the green light in BGR (23% green) and GR (41% green) lights enhanced Chl a and b accumulation by ~41% under limited N availability.

Further, the chlorophyll concentration in spinach leaves under BGR and GR lights was significantly higher than in BR, implying additive impacts of green light on chlorophyll synthesis. Although the quantum efficiency of PSII (PhiPS2) or Q_Y was unchanged under three spectra, the electron transport rates were significantly higher (77% under high N and 50% under low N) in BGR and GR than in BR alone. Green light contributes to photosynthesis more efficiently than red or blue light due to the non-photosynthetic absorption of green light by carotenoids (Mccree, 1971), which was confirmed in our experiment where carotenoids were significantly higher in BGR (23% green) and GR (41% green) and also showed higher shoot and root fresh and dry biomass under limited N availability. The shoot and root biomass under BGR were significantly higher than BR or GR when the N was limiting. The higher ETR and chlorophyll accumulation are consistent with studies that demonstrated deeper penetration of green light into leaves to assimilate CO_2 efficiently (Brodersen and Vogelmann, 2010; Liu and Van Iersel, 2021).

Our results showed a significant reduction in stomatal conductance under low N availability in BGR and GR lights. It has been suggested that lower stomatal conductance could result from stomata responding directly to signals induced by N deprivation (Broadley et al., 2001). Intriguingly, stomatal conductance was not affected in BR (4% green) due to N on and was significantly higher than BGR (23% green) or GR (41% green) when N was limiting, which was consistent with studies in lettuce that showed the highest stomatal conductance under red and blue but lowest in the presence of green lamps (Kim et al., 2004). Green light has been reported to inhibit blue-light-induced stomatal opening (Talbott et al., 2002; Folta and Maruhnich, 2007; Matthews et al., 2020). We observed that the dry shoot and root biomass of plants grown in BGR were significantly higher than BR or GR under lower N. It is plausible to assume that supplemental green light in GR and supplemental red light in BR could negatively impact biomass under limited N. On the other hand, consistent with prior reports (Thi et al., 2020), additional green light in BGR compared to BR could have positively altered productivity and quality in spinach.

Frequent and substantial N applications are typical in traditional or indoor production systems. Optimization of N supply and its efficient management is critical to productivity, quality, and cost-prohibitive for indoor vegetable production. Increasing N supply enhances productivity and N content at the expense of NUE (Zhang et al., 2015; Frerichs et al., 2022). Although environmental groundwater contamination due to leaching under CEA is less of a concern, studies have shown that lowering N application increases NUE and reduces N losses (Song et al., 2009). The higher NUE under high or low N availability could result from saturated light in BGR or supplemental green light in GR compared to BR alone. Although research on the impact of different lights on NUE in spinach is still limited, higher NUE under the limited N we observed is consistent with other field-based or hydroponic studies in spinach (Canali et al., 2014; Zhang et al., 2015; Chan-Navarrete et al., 2016). Nevertheless, either a positive role of blue light on N allocation from root to shoot, only under limited nitrogen (Liang et al., 2022) or no significant impact on N uptake or utilization due to red or blue lights or their combinations (Clavijo-Herrera et al., 2018; Pennisi et al., 2019a; Pennisi et al., 2019b) has also been shown in lettuce.

In the present study, all light spectra demonstrated typical OJIP phases, indicating that the plants were photosynthetically active. Our results confirmed that nitrogen-induced changes in OJIP parameters are susceptible to any change in PSII activity, where light spectra efficiently impacted PSII activity in nitrogen stress. The OJIP curve shows changes associated with reducing the primary electron acceptor of photosystem II (PSII) and the efficiency of electron transport. The spectral composition alters the photosynthetic efficiency, the distribution of excitation energy between PSI and PSII, and the balance between photochemical and non-photochemical quenching mechanisms, leading to variations in the amplitude, kinetics, and shape of the OJIP curve. Unlike GR or BGR, higher fluorescence intensity in BR suggests higher absorption of red light by chlorophyll a and b, promoting the excitation of electrons in PSI and PSII, leading to a distinct OJIP curve shape with rapid transitions between the O, J, I, and P steps. Rapid transitions induced in the OJIP curves due to red light are consistent with studies in other species (Costa et al., 2021).

The data demonstrated that the most predominant amino acids in spinach leaves were major N-rich transporting amino acids such as glutamine, glutamic acid, aspartic acid, GABA, alanine, and serine. Among the major amino acids, the percent accumulation of glutamine and aspartic acid was significantly reduced under limited N. On the other hand, excepting alanine, GABA, and serine under BR, the percent accumulation of most amino acids remained the same irrespective of N treatment under all lights. As demonstrated by PCA analysis, most N-rich amino acids were clustered with high N in leaf tissue but did not respond to the light spectrum significantly, signifying light-induced changes in their accumulation were less pronounced in spinach leaves. Intriguingly, the negative association of glutamic acid, aspartic acid, and BR with glutamine, BGR, and GR in root tissues implicates the role of the light spectrum in the partitioning and assimilation of amino acids. Unlike reports in lettuce (Bian et al., 2018), adding green light to continuous red and blue light did not significantly alter the nitrate or total N content in spinach under sufficient nitrogen but showed an increase when N was limited. Consistent with other studies (Bian et al., 2018), we speculate that the additional green light in BGR, along with relative contributions of blue and red light, may have collectively resulted in enhancing NR and GDH activities under high N and GDH, GOGAT and GS activities compared to BR, under limited N.

The varied mineral composition under different light spectra in our data was consistent with other studies using white, red, and blue lights in spinach (Thi et al., 2020) and red, blue, and green lights in lettuce (Razzak et al., 2022) and microgreens (Kamal et al., 2020) or red and blue (Brazaitytė et al., 2021) in microgreens. Iron accumulation in spinach was also reported to be affected by its concentration in hydroponic solution rather than by the ratio of red to blue lights (Vaštakaitė-Kairienė et al., 2022). The positive association between nitrogen and oxalate in spinach was in agreement with several other studies (Ota and Kagawa, 1996; Zhang et al., 2005; Solberg et al., 2015; Joshi et al., 2021). Although similar to other reports (Gao et al., 2020), the oxalates under BR were significantly lower than BGR under high N, and unaffected oxalate accumulation under low N in BR suggests the possibility of N-independent regulation of its synthesis in spinach.

5 Conclusion

Taken together, the research underscores the dynamic interplay between light quality and nitrogen availability in modulating spinach productivity and quality. The shoot biomass of spinach was least influenced by additional green light but showed significant increases in the root fresh biomass when the nitrogen was limited. The impacts of green light (GR and BGR) showed additive effects based on higher chlorophyll concentrations, electron transport rates, and higher activities of nitrogen assimilatory enzymes (GDH, GOGAT, GS, and NR). The positive impacts of green light were more apparent when nitrogen was limited, based on higher accumulation of chlorophylls (a and b) and percent accumulation of glutamic acids or glutamine. This study provides new insights into regulating biochemical and physiological aspects under different light spectra in spinach. Additional research involving global expression analysis and manipulation of the relative contribution of light intensity and spectral distribution would help elucidate unknown nitrogen-associated mechanisms.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

MR: Formal analysis, Investigation, Methodology, Software, Writing – original draft, Data curation. DT: Data curation, Formal analysis, Methodology, Writing – original draft. MM: Formal analysis, Methodology, Writing – original draft, Investigation. VJ: Formal analysis, Investigation, Methodology, Writing – original draft, Conceptualization, Funding acquisition, Project administration, Resources, Software, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1283730/full#supplementary-material

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EDITED BY
Giao Nguyen,
Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia

REVIEWED BY
Kristina Laužikė,
Lithuanian Research Centre for Agriculture and Forestry, Lithuania
Sandra Saunoriūtė,
Vytautas Magnus University, Lithuania

*CORRESPONDENCE
Józef Sowiński
jozef.sowinski@upwr.edu.pl

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The effect of peat replacement in horticulture media by willow (*Salix viminalis* L.) biomass compost for cucumber transplant production

Katarzyna Adamczewska-Sowińska[1], Józef Sowiński[2*], Elżbieta Jamroz[3] and Jakub Bekier[3]

[1]Department of Horticulture, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland, [2]Institute of Agroecology and Plant Production, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland, [3]Institute of Soil Sciences Plant Nutrition and Environmental Protection, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

This research evaluated the usefulness of horticultural substrates prepared on the basis of compost from chipped willow without additives and with the addition of nitrogen and decomposing mycelium of the cellulose-lignin fraction of wood in the cultivation of cucumber seedlings. The produced composts were mixed in different proportions: mixture 1 (W1) - the proportion of compost without additives and compost prepared with the addition of nitrogen and mycelium was in the ratio of 50:50, mixture 2 (W2) - the proportion of compost without additives and compost prepared with the addition of nitrogen and mycelium was in the ratio of 75:25. The starting mixtures were used to prepare horticultural substrates with different components (peat - P, vermicompost - V) and additives: basaltmeal - B, biochar from deciduous wood - C. The components were added in varying proportions. A total of 29 different substrates were subsequently tested in the study. Plant showed that the traits assessed varied to a greater extent under the effect of the test factors than at earlier growth stages. It was demonstrated that cucumber grown on substrates with 75% or 50% willow compost had a unit weight at the same statistical level as when grown on peat substrate (P). The plants with the highest unit weight (8.5- 10.4 g), belonged to the same homogeneous group and derived from sites W1P1B2, W2P1, W1P1B1, W2P2, W1P1C1, P, W1P1, W2B1, W2P2B2. High-quality cucumber transplant should characterise well develop, optimal height-to-stem thickness ratio, short hypocotyl, thick green leaves and cotyledons.

KEYWORDS

alternative horticulture media, mixture proportion, cucumber, transplant parameters, willow

Introduction

Peat, a widely used substrate component in horticulture, has faced criticism due to environmental concerns and ongoing climate change (Hong and Gruda, 2020; Gruda, 2021). For example over the past decade (2011-2021), the proportion of peat in total horticultural media in the UK has decreased by 26 percentage points (pp) and currently accounts for 36% of the total (Holmes and Bain, 2023). Therefore, the quest for peat-based substrate additives or alternatives to peat for new horticultural applications has intensified. In this regard, research is underway on environmentally safe composts, biochar, agricultural waste compost, and vermicompost (Gruda et al., 2009).

Earlier studies have shown the suitability of cellulose-lignin biomass composts from willow (*Salix viminalis* L.) for the production of substrates used in tomato and cucumber transplant cultivation (Adamczewska-Sowińska et al., 2021; Adamczewska-Sowińska et al., 2022). The carbon content of willow biomass ranges from 502-530 g per kg dry weight while the nitrogen content ranges from 3.9 to 7.5 g per kg dry weight (Krzyżaniak et al., 2015; Liu et al., 2016; Weger et al., 2016; Larsen et al., 2019; Stolarski et al., 2020a). The broad carbon:nitrogen ratio in biomass of 125:1 based on data presented by Stolarski et al. (2020b) needs to be corrected in order to start the biomass composting process properly. An appropriate C:N ratio is a very important parameter influencing the growth of microorganisms and the optimum ratio for their development is considered to be in the range of 25-35. One that is too high (above 35:1) may lengthen the biotransformation period and one that is too low (below 20) increases nitrogen losses due to faster mineralisation of biomass (Bernal et al., 2009; Osono, 2015; Arnstadt et al., 2016; Akratos et al., 2017).

By using alternative organic materials in horticultural substrates (such as compost, wood or plant fibre), microbial biomass may increase and contribute to reduced compost stability (Agarwal et al., 2021; Vandecasteele et al., 2022). In addition, preliminary sanitation treatments are needed to ensure the destruction of weed seeds, pathogens. It is recommended to carry out treatments such as acidification or thermal phase during composting before preparing the substrates (Grunert et al., 2016; Vandecasteele et al., 2018). During the composting process, increasing the temperature to 35-40°C also promotes microbial biodiversity (Storey et al., 2015), while further temperature elevation to 45-55°C enhances the rate of biomass biodegradation and accelerates mineralisation (Ryckeboer et al., 2003). The addition of mineral nitrogen provides optimum conditions for composting of willow biomass (Sowiński et al., 2022). Too high a nitrogen content can cause salinisation of the substrate and have a negative effect on the growth of tomato plant transplants (Adamczewska-Sowińska et al., 2021).

The use of composts as a major substitute for peat or coconut fibre and the provision of optimum parameters is an essential requirement to be met in the preparation of a good horticultural growing medium (Vandecasteele et al., 2021). The optimum pH, salinity and nutrient content are vital. Meeting these parameters requires the selection and blending of suitable composts or a variety of both organic and mineral materials (Vandecasteele et al., 2021). In a study by Giménez et al. (2020), replacing peat as a growing medium component in leafy vegetable production with 25% compost had a beneficial effect on yield and crop quality. Similarly, Tüzel et al. (2020) found that compost from olive production waste could replace peat in the mixture as a substrate for tomato seedling production in 25%. Replacing peat with vermicompost or biochar did not have a negative effect on horticultural plant growth (Alvarez et al., 2017).

The primary goal of the research was to obtain a new substrate that would allow the production of cucumber seedlings with quality parameters similar to those in peat substrate. The aim of the study was to find answers to the question of the quality of cucumber seedlings produced in substrates prepared based on composts from willow biomass, combined in various proportions with peat or vermiculite, as well as with additions of biochar or basalt meal.

On the basis of the formulated objective, the research hypotheses were formulated: a mixture of willow composts will provide similar conditions for the growth of cucumber seedlings as peat substrate. The addition of milled basalt rock, vermicompost and biochar will improve the parameters of cucumber seedlings and significantly reduce the use of peat in seedling production and may improve growing conditions.

Methodology

In 2019, four willow chip-based composts were produced: compost from willow chips alone and compost with the addition of nitrogen and the wood-decomposing mycelium *Peniophora gigantea* of the Mycelium and Biopreparations Factory 'POSZWALD'. The favourable effect on the composting process by the additives used was due to the broad C:N ratio of 118:1 in the willow biomass (Adamczewska-Sowińska et al., 2021).

Two mixtures with different proportions of compost (volume ratios) were prepared (Table 1):

- in mixture 1 (hereafter referred to as W1), the proportion of compost without additives and compost prepared with nitrogen and mycelium was 50:50.
- in mixture 2 (hereinafter referred to as W2) - the proportion of compost without additives and compost prepared with nitrogen and mycelium was 75:25.

Horticultural substrates for growing cucumber seedlings were prepared using mixtures W1 or W2 with different additives. These were.

- peat (P), the proportion of which varied and was 50, 25 and 10% in volume percentage, referred to as P1, P2, P3 respectively.
- vermicompost (V), the proportion of which varied and in volume percentages was 50, 25 and 15%, labelled V1, V2, V3 respectively.

TABLE 1 Proportion of individual components in the tested media.

Code	Horticulture media proportion (% of volume capacity)			Additives (g per 100 g of horticulture media)	
	willow compost	peat	Vermicompost	basaltmeal	biochar
Media component and their percentage					
P	–	100	–	–	–
V	–	–	100	–	–
W1	100	–	–	–	–
W2	100	–	–	–	–
W1P1	50	50	–	–	–
W1P2V2	50	25	25	–	–
W1V1	50	–	50	–	–
W2P1	50	50	–	–	–
W2P2V2	50	25	25	–	–
W2V1	50	–	50	–	–
W2P2	75	25	–	–	–
W2P3V3	75	10	15	–	–
W2V2	75	–	25	–	–
Media with additives					
W1B1	100	–	–	5	–
W1B2	100	–	–	10	–
W1P1B1	50	50	–	5	–
W1P1B2	50	50	–	10	–
W2B1	100	–	–	5	–
W2B2	100	–	–	10	–
W2P2B1	75	25	–	5	–
W2P2B2	75	25	–	10	–
W1C1	100	–	–	–	10
W1C2	100	–	–	–	20
W1P1C1	50	50	–	–	10
W1P1C2	50	50	–	–	20
W2C1	100	–	–	–	10
W2C2	100	–	–	–	20
W2P2C1	75	25	–	–	10
W2P2C2	75	25	–	–	20

Two groups were separated for substrate preparation and mineral additives of milled basalt rock and biochar were applied to one.

- milled basalt rock meal (basaltmeal) (B) at 5 g per 100 g of substrate - B1, and 10 g per 100 g of substrate - B2.
- biochar prepared from waste deciduous wood (C) at 10 g per 100 g of substrate - C1, and 20 g per 100 g of substrate - C2.

In 2021, experimentation on the obtained media commenced. On 7 April, cucumber seeds of the Hermes Skierniewicki F1 cultivar were spot sown into pots filled with the prepared growing media. Each variant with horticultural media was repeated 10 times.

Biometric measurements of cucumber plants were conducted on the dates April 20, April 27, and May 4, respectively, 13, 20, and 27 days after the seed sowing date. On 10 plants from each combination, height and lateral extent were assessed using a measuring tool with an accuracy of 0.1 cm, along with the leaf count. Based on these measurements, the growth increment of each parameter between the mentioned measurement dates was calculated, contributing to the characterization of the plant growth rate. In the last measurement session, before terminating the experiment, the stem diameter was also evaluated at a height of 0.5 cm from the plant base, and the individual plant mass was determined. The ratio of plant height to stem diameter was calculated – an index describing the condition of the plants. Electronic calipers and a precision electronic scale (accuracy 0.1 g) were used for these measurements.

Statistical analysis

Data from the morphological measurements of plants and the quantitative and qualitative evaluation of seedlings collected at individual dates and before harvest were subjected to ANOVA/MANOVA analysis in Statistica software (version 13.1, StatSoft, Poland). All analyses were performed at a significance level of $p < 0.05$. 1- and 2-way analysis of variance was performed to assess the effect of medium type and different proportions of components in the medium mixture on the determined cucumber seedling parameters. The substrates and proportions in the mixture corresponded to the fixed effect of the model, while the repetitions accounted for the variable effect of the model. The analysis assessed the effect of the proportion of willow compost, peat, vermicompost, basaltmeal and biochar as a substrate component as compared to homogeneous media.

Results

Thirteen days after sowing the seeds, the majority of cucumber seedlings across the different sites exhibited a similar stage of development, characterized by well-developed and distributed cotyledons and the appearance of the first true leaf. Between 90 - 100% of the seedlings had the first true leaf: W2P2, W2V2, W2B1, W2P2B2, W1P1C1, W2C2 and W2P2C2, and 88 - 89% at sites W2C1 and W1C1 (Tables 2 and 2A). Conversely, the fewest plants with the first leaf were recorded in sites: W1, W1P2V2 (33 - 44%), W2B2, W2P2B1 (50%) and V and P (56%).

As shown in Tables 2 and 2A, at the beginning of growth, the type of growing medium had no significant effect on the number of leaves in cucumber plants. It ranged from 0.3 - 0.4 leaves per plant at sites W1 and W1P2V2 to 0.8 leaves per plant (W2, W2V1, W2P3V3, W1V1) and 0.9 pcs. (W2P2, W2V2). The lateral extent of the plants was observed to vary significantly, ranging from 5.8-6.5 cm (W1, V, W2P2V2) to 8.0-8.2 cm (W2V2, W1P1, W2P2). There was, however, a noticeable tendency for plants growing in peat medium or in media with peat as a component compared to those growing in basal W1 and W2 to have a greater spread. Cucumber plants grown in W2 substrate had significantly greater height (by 25.9%) and 2.7 times more leaves compared to W1.

Notably, the greatest heights were those of plants (4.4 cm) from sites W2P3V3 and W2P2 (4.3 cm). In the same homogeneous group was the height of plants from V (4.0 cm), W2 (3.7 cm) and W1P1 (3.7 cm). In contrast, the addition of more vermicompost (V1) to the compost of W1 and W2 resulted in plants in a group with significantly lower height.

TABLE 2 The effect of horticultural media on cucumber transplant at 13 Days After Sowing (DAS).

Code	Lateral extent (cm)	Height (cm)	Average leaves number
P	6.9abcde*	3.4bcd	0.6
V	6.1ab	4.0de	0.6
W1	5.8a	3.3bcd	0.3
W2	7.3bcde	3.7cde	0.8
W1P1	8.1de	3.7cde	0.7
W2P1	6.7abc	2.8ab	0.7
W2P2	8.0cde	4.3e	0.9
W1V1	6.8abcde	2.9abc	0.8
W2V1	7.1abcde	2.4a	0.8
W2V2	8.2e	3.3bcd	0.9
W1P2V2	6.7abc	3.3bcd	0.4
W2P2V2	6.5ab	2.8ab	0.6
W2P3V3	7.1abcde	4.4e	0.8
Significance	**P<0.05**	**P<0.001**	**n.s.**

*Means in the same column that include a lowercase letter are not statistically significant.
n.s., no significant.

The addition of basaltmeal (B1 or B2) or peat together with a higher dose of basaltmeal (P1B2) to the W1 growing medium increased plant lateral extent by an average of 20.7%, but this difference was not confirmed statistically (Table 2A). However, cultivation on W1P1B1 substrate resulted in cucumber plants with the largest lateral extent (8 cm) and being among the tallest with the largest number of leaves (average 0.8). Plants grown on W2B1 and W2P2B2 substrate were also exhibited substantial growth. The addition of peat or basaltmeal to the W2 substrate did not result in significant changes in the measured cucumber plant traits.

The observations revealed that the addition of biochar to the W1 substrate at a lower dose (C1) led to a notable trend towards a 20% increase in plant extent, although this trend was not statistically confirmed. However, the incorporation of peat (P1C1) resulted in a significant increase of 37.9% in plant lateral extent. No changes were observed in plant height, while the number of leaves on average increased by more than three times. In contrast, the addition of biochar to the homogeneous W2 substrate or to its mixture with peat did not produce any significant changes in the measured cucumber plant traits.

On the second observation date, which was at 20 DAS, it was observed that the type of substrate did not have a significant effect on the lateral extension of the cucumber plants or their foliage, as shown in Table 3. The lateral extension of the plants ranged from 8.9 cm for W2V1 to 12.8 cm for W1P1. However, there was a noticeable tendency for plants grown in peat substrate (P) or substrates containing peat (W1P1, W2P1, W2P2) to exhibit increased lateral extent compared to those grown in basic

TABLE 2A The effect of horticultural media additives on cucumber transplant quality at 13 DAS.

Code	Lateral extent (cm)	Height (cm)	Average leaves number
P	6.9cdef*	3.4abcd	0.6abc
W1	5.8abc	3.3abc	0.3a
W2	7.3defg	3.7abcde	0.8bcd
W1B1	7.0cdefg	3.2abc	0.8bcd
W2B1	8.2g	4.0bcde	0.9cd
W1B2	7.0cdefg	3.4abcd	0.6abc
W2B2	6.7bcde	3.4abcd	0.5ab
W1P1B1	8.0fg	4.5e	0.8bcd
W1P1B2	7.0cdefg	4.1cde	0.6abc
W2P2B1	6.1abcd	4.1cde	0.5ab
W2P2B2	7.8efg	4.2de	1.0d
W1C1	6.9cdef	3.0a	0.9cd
W2C1	7.0cdefg	4.0bcde	1.0d
W1C2	5.4a	3.4abcd	0.6abc
W2C2	6.6abcd	4.1cde	1.0d
W1P1C1	8.0fg	3.4abcd	1.0d
W1P1C2	5.5ab	3.7abcde	0.8bcd
W2P2C1	6.6abcd	4.0bcde	0.9cd
W2P2C2	7.0cdefg	3.4abcd	0.9cd
Significance	**P<0.001**	**P<0.05**	**P<0.01**

*Means in the same column that include a lowercase letter are not statistically significant.
n.s., no significant.

substrates W1 and W2. In addition, it was found that plants in these sites were on average 13.8% taller. The use of vermicompost as a homogeneous substrate or substrate component did not enhance cucumber growth, with the exception of the response to W2P3V3 substrate, which contained the lowest amount of vermicompost. The plants grown in this substrate belonged to the group with the greatest height (10.2 cm) and were distinguished by their spread (11.2 cm) and number of leaves (average 1.7).

The statistical analysis of the experimental data confirmed that the addition of basaltmeal or biochar to the W1 or W2 substrate mixed with peat had a significant effect on the cucumber plant quality (refer to Table 3A). In the WPB media treatment, the lateral extent of the plants increased by an average of 20.9% compared to the plants grown in WB, while in WPC, the lateral extent increased by 11.2% compared to those in WC. Moreover, the foliage of WPB plants was on average 12.3% larger than in WB.

The best-performing plants in terms of spread and number of leaves were obtained from sites W1P1B1, W1P1B2, W2P2B2 and W1P1C1. It was also observed that a similar lateral extent was determined in plants grown in W2B1, W2P2B1, W2P2C1, and W2P2C2 media. Furthermore, plants from media W2B1 (11.2 cm), W1P1B1 (11.7 cm), and W1P1B2 (11.5 cm) had a significantly greater height than those in other sites, with an average increase of 38.4%.

TABLE 3 The effect of horticultural media on cucumber transplant quality at 20 DAS.

Code	Lateral extent (cm)	Height (cm)	Average leaves number
P	12.0	8.2abc*	1.6
V	9.8	8.8abcd	1.3
W1	9.2	8.0abc	1.6
W2	9.6	8.7abcd	1.8
W1P1	12.8	8.9bcde	1.8
W2P1	10.3	9.2cde	1.7
W2P2	11.0	10.4e	1.6
W1V1	9.8	8.3abc	1.7
W2V1	8.9	8.4abc	1.5
W2V2	11.2	9.1bcde	1.6
W1P2V2	9.8	7.6ab	1.6
W2P2V2	10.3	7.1a	1.6
W2P3V3	11.2	10.2de	1.7
Significance	**n.s.**	**P<0.01**	**n.s.**

*Means in the same column that include a lowercase letter are not statistically significant.
n.s., no significant.

The biometric measurements conducted prior to the termination of the experiment revealed that the lateral extent and height of plants were significantly influenced by the substrate type (Table 4). Conversely, the number of leaves per plant, which ranged from 2.5 to 3.2, did not differ significantly among the treatments. The W2P2 substrate, consisting of 75% willow compost (W2 mixture) and 25% peat, resulted in the most vigorous seedlings, exhibiting the greatest lateral spread (27.3 cm) and height (15.5 cm). Plants grown in peat substrate and in W2P3V3 and W2V2 substrates showed similar lateral spread at a significant level, whereas height was found to be significant only in the W2P3V3 treatment. Furthermore, a trend towards reduced plant biomass was observed with an increase in the proportion of vermicompost in the substrate. Specifically, when vermicompost constituted 50% of the mixture with willow compost, the average lateral spread and height of plants were 20.4% and 9.2% lower, respectively, compared to the 25% vermicompost mixture. Similarly, when vermicompost accounted for 25% of the mixture with willow and peat, the average lateral spread and height of plants were 11% and 16.5% lower, respectively, compared to the 15% vermicompost mixture.

Cucumber plant responses to the addition of basaltmeal to the tested substrates were analysed, and the results are presented in Table 4A. The research revealed that the addition of basaltmeal to the W1 substrate did not lead to improvements in basic quality characteristics when present in either lower or higher doses. However, it was found that the addition of basaltmeal to the W1 mixture with peat had a positive effect on the seedlings, resulting in

TABLE 3A The effect of horticultural media additives on cucumber transplant quality at 20 DAS.

Code	Lateral extent (cm)	Height (cm)	Average leaves number
P	12.0e*	8.2abcde	1.6abcd
W1	9.2abc	8.0abcd	1.6abcd
W2	9.6abcd	8.7bcdef	1.8d
W1B1	8.5a	8.1abcd	1.4a
W2B1	11.7de	11.2g	1.5ab
W1B2	8.4a	7.8abc	1.4a
W2B2	8.8ab	8.8bcdef	1.4a
W1P1B1	12.2e	11.7g	1.7cd
W1P1B2	11.1bcde	11.5g	1.6abcd
W2P2B1	10.1abcde	8.9cdef	1.4a
W2P2B2	11.6de	9.2def	1.7cd
W1C1	9.2abc	7.7abc	1.4a
W2C1	9.6abcd	9.6ef	1.4a
W1C2	8.2a	6.8a	1.5ab
W2C2	8.8ab	8.8bcdef	1.4a
W1P1C1	11.5cde	9.6ef	1.6abcd
W1P1C2	8.1a	6.8a	1.4a
W2P2C1	10.2abcde	8.2abcde	1.4a
W2P2C2	10.0abcde	7.5ab	1.5ab
Significance	**P<0.001**	**P<0.001**	**P<0.05**

*Means in the same column that include a lowercase letter are not statistically significant. n.s., no significant.

TABLE 4 The effect of horticultural media on cucumber transplant quality at 27 DAS.

Code	Lateral extent (cm)	Height (cm)	Average leaves number
P	27.0de*	11.3ab	3.0
V	23.1bc	12.3abc	2.7
W1	21.1abc	11.4ab	2.7
W2	23.0bc	11.3ab	3.0
W1P1	23.1bc	12.7bc	2.9
W2P1	23.4bcd	13.0cd	2.8
W2P2	27.3e	15.5e	3.0
W1V1	18.1a	10.9a	2.6
W2V1	20.6ab	11.8abc	2.5
W2V2	24.3bcde	12.5bc	3.2
W1P2V2	21.6abc	12.1abc	2.8
W2P2V2	22.4bc	11.6abc	2.9
W2P3V3	24.7cde	14.2de	2.9
Significance	**P<0.001**	**P<0.001**	**n.s.**

*Means in the same column that include a lowercase letter are not statistically significant. n.s., no significant.

greater lateral extent by an average of 26.3% and increased plant height by 30% on average. Conversely, no significant variation in seedling characteristics was observed when W2 was the basic component of the growing medium. The best-quality seedlings were obtained on W1P1B1, W2P2B2, and W2B1 growing media. These plants exhibited lateral extent similar to those grown in peat substrate and, on average, 23.9% greater height and 10% more leaves.

The results showed that the addition of biochar did not result in improvements in seedling quality. In the W1C2 media, the plant lateral extent and height, as well as the number of leaves, were the lowest among plants grown on substrates containing biochar. However, it was observed that the addition of biochar to substrates consisting of willow compost and peat promoted plant growth, especially when the addition of biochar was lower. The cucumber seedlings growth achieved on W1P1C1 and W2P2C1 substrates, matched the quality of plants grown in peat substrate. Moreover, these substrates were characterised by greater height and slightly better foliage at the W1P1C1 media.

The analysis of the experimental results revealed that cucumber plants, in their third week of vegetation, exhibited a faster rate of vertical growth compared to lateral growth, irrespective of the substrate type or additive used (see Figures 1A, B and 2A, B). However, in the fourth week, a relatively smaller increase in plant height compared to lateral extent was observed. Between the first and second measurement dates, plants grown on W2P1 and W2V1 substrates, as well as those grown on W1V1 and W2P2V2 substrates, exhibited a rapid increase in plant height. Between the second and third measurement dates, the plants grown on W2P2V2 and W1P2V2 substrates exhibited the most rapid increase in plant height. Plants from W2P1 and W2V1 media showed a six-fold faster increase in height in the third week than in the fourth week, while those from W2P2V2 and W1P2V2 media grew only 1.9-2.8 times faster.

The results of the experiment indicate that in the fourth week of vegetation, the plants from sites V, W2P2, W2V1, and W2P1 demonstrated the highest lateral extent. However, the rate of lateral extent growth compared to week 3 was significantly higher on the W2V1, W2P2, and W2 substrates (4.9 - 4.1 times faster) compared to the plants from the other sites (1.5 - 3.1 times faster).

On completion of the third week of cucumber vegetation, it was observed that the addition of 10% (w/w) milled basalt to the W1 medium had a retarding effect on the growth of plant height compared to 5% (w/w). Conversely, the incorporation of the same quantity of basaltmeal into the W1 mixture with peat resulted in an acceleration of plant growth, with an increase in plant height of 180.5%. This increase was one of the highest observed. Additionally, a similar increase in plant height was observed in those cultivated on the W2 substrate with the addition of a 5% dose of basaltmeal.

The incorporation of 20% (w/w) biochar, into the W1 or W2 compost substrate and the W1P1 and W2P2 mixture led to a decrease in the rate of seedling vertical growth. However, the W1P1C1 site was an exception, where the height gain between I

TABLE 4A The effect of horticultural media additives on cucumber transplant quality at 27 DAS.

Code	Lateral extent (cm)	Height (cm)	Average leaves number
P	27.0gh*	11.3bcd	3.0bcde
W1	21.1bcd	11.4bcde	2.7abc
W2	23.0def	11.3bcd	3.0bcde
W1B1	19.6abc	11.8cde	2.8abc
W2B1	25.4efgh	13.4fg	3.3def
W1B2	17.3a	10.5ab	2.6ab
W2B2	22.6cde	10.7abc	2.8abc
W1P1B1	28.4h	15.2h	3.1cdef
W1P1B2	24.9efg	14.2gh	2.9abcd
W2P2B1	22.4cde	11.7bcde	2.8abc
W2P2B2	25.3efgh	13.4fg	3.5f
W1C1	22.9def	10.4ab	2.8abc
W2C1	24.1defg	12.5def	2.8abc
W1C2	19.1ab	9.7a	2.5a
W2C2	21.5bcd	11.7bcde	2.8abc
W1P1C1	27.2gh	12.6ef	3.3def
W1P1C2	23.1def	10.9abc	2.7abc
W2P2C1	25.8fgh	11.8cde	3.0bcde
W2P2C2	24.3defg	11.0bc	3.1cdef
Significance	**P<0.001**	**P<0.001**	**P<0.001**

*Means in the same column that include a lowercase letter are not statistically significant. n.s., no significant.

and II measurement was 182.4%. Between the second and third measurement dates, the height increase of cucumber seedlings was observed to be 1.4-2.6 times slower in W1P1C2, W1C2, W2P1C1, and W2P1C2 and 7.4-9.2 times slower in W2B2, W1P1B2, and W2B1 than the first and second measurement periods. The largest height increase of 60.3% was recorded in plants growing in W1P1C2.

After a three-week growth period of cucumber plants, no significant effect was observed on the rate of lateral reach growth upon the addition of basaltmeal to W1. Nevertheless, it was noted that the presence of a lower dose of basaltmeal at W2B1 and, especially, at W2P2B1 resulted in a faster increase in plant spread. At the last measurement date, only seedlings from W2B2 had a faster lateral growth rate than plants on the W2 basic substrate.

The addition of biochar to the W1 substrate significantly enhanced the lateral spread of the seedlings by the end of the production period compare o previous measurement term at W1C1 (148.9%) and W1P1C2 (185.2%) media. Transplants cultivated at W1B1, W1B2, and W2B2 exhibited distinctively faster lateral growth gains between three-week-old and four-week-old seedlings, with a 5-6.1-fold increase compared to W2P2B1 (a 1.9 fold).

Upon evaluating the effect of substrate type on shoot diameter, it was determined that cucumber seedlings grown on peat substrate and W2P2 had the largest diameter (5.9 mm) (see Figure 3). Transplants cultivated at W1P1, W2P1, and W2P3V3 exhibited a similar diameter and were also included in the same homogeneous group. The smallest shoot diameter was observed in plants from sites W1, W2, W1V1, and V (4.4-4.8 mm). Addition of peat to W1 and W2 substrates resulted in a statistically significant average increase in plant diameter of 24.6%. Mixing W2 compost with vermicompost in ratios of 50:50 or 75:25, and W2 compost with peat and vermicompost in a ratio of 50:25:25, resulted in an insignificant increase in shoot diameter by an average of 8.3%. However, altering the ratio in the W2P3V3 mixture to 75:10:15 resulted in a significant increase in diameter by 12.5% compared to W2.

The stem height:diameter ratio was determined by calculating the ratio of plant height to shoot diameter at the base and was statistically analysed (see Figure 3A). The plants grown in peat substrate had the lowest index (19.4), whereas those from homogeneous substrates W2, V, and W1 were significantly more stocky, with indices of 23.8, 25.5, and 26.3, respectively. The index from sites W1P1 and W1V1 fell into the same homogeneous group. On average, decreasing the percentage of peat in the substrates from 50%, to 25%, and 10% increased the index values to 23.4, 24.8, and 26.7. The stem height:diameter index also increased with decreasing the percentage of vermicompost in the substrates from 23 and 24 (50 and 25% share) to 26.7 (15% share), on average.

Upon statistical analysis, it was demonstrated that the addition of basaltmeal to the compost and peat mixture led to a 19.9% increase in the average plant diameter, which was equivalent to that of plants grown in pure peat substrate (as illustrated in Figure 3B). Conversely, the plants grown in substrates with added biochar exhibited a differential response. In this case, only the addition of a smaller dose of this component to the homogeneous compost substrate or to the compost and peat mixture resulted in a statistically significant increase in shoot diameter compared to W1 and W2. Only at the W1PC1 site (5.8 mm) did the plants reach the same diameter as those grown in pure peat (5.9 mm). Furthermore, a statistical comparison of the stockiness coefficients showed lower values for seedlings grown in media with added biochar, compared to plants from facilities where basaltmeal was utilized as an additive.

The study revealed that cucumber plants grown on substrates containing a combination of willow compost and peat in a ratio of 75% or 50% attained similar unit weights to those grown on pure peat substrate (Figure 4A). Plants from the W2P2V2 and W2P2 sites had statistically similar weights (10.1 g). In contrast, the addition of 50% vermicompost or pure willow compost to the substrate resulted in an average of 1.5 times lower plant weight.

Furthermore, the study found that the addition of basaltmeal at a rate of 5% (w/w) of substrate to W1 or W2 resulted in a 31.3% increase in plant weight (Figure 4B). When basaltmeal was added to compost-peat mixtures at a rate of 5% or 10% (w/w) of substrate, plant weight was almost twice as high (W1P1B1 and W1P1B2) or 1.2 times higher (W2P1B1 and W2P1B2) than the weight of plants grown on base compost substrates (W1 and W2). The addition of biochar to the substrates also had a beneficial effect on plant weight, resulting in an 84.9% and 14.9% increase in weight on W1PC1 and W2PC1, respectively, compared to plants grown on W1 and W2. The combined use of both additives had a favourable impact in the case of media containing peat as a component.

The findings show that cucumber transplants cultivated on W1 substrate with 50% of both initial composts exhibited a more pronounced response to the additives as compared to those grown in W2 substrate, as indicated in Figure 5. The results demonstrated that the inclusion of peat in the substrates led to the greatest increase in plant weight, by 81.1% and 50.7%, respectively. Moreover, the addition of basaltmeal to the substrates containing W1 and W2 resulted in a significant increase in plant unit weight of 34% and 31.3%, respectively, whereas the addition of biochar to the compost mixture with W1 led to a 24.5% increase in plant weight.

Discussion

Among alternative material, willow biomass compost mixtures are a promising type of plant biomass that can significantly replace environmentally valuable peat. In UK conditions, wood-based raw materials are the primary alternative to peat (Holmes and Bain, 2023). In a previous study (Sowiński et al., 2022), willow composts were found to have no toxic effect on white mustard seed germination, and some aqueous extracts from compost-peat mixtures even showed a stimulating effect on white mustard seedlings. Composts made from cellulose-lignin biomass of willow (*Salix viminalis* L.) have shown suitability of for the production of horticulture media used in tomato and cucumber transplant as well as for lettuce cultivation (Adamczewska-Sowińska et al., 2021; Adamczewska-Sowińska et al., 2022; Bekier et al., 2022).

It was found that a substrate mixture (W2) composed of compost without additives and compost with added nitrogen and mycelium in a ratio of 75:25 was more effective than a mixture with an equal proportion of both composts (W1). In a study by Adamczewska-Sowińska et al. (2021), demonstrated that the C:N ratio in biomass is crucial not only during the willow composting process but also in seedling growth. The use of nitrogen-added homogeneous substrate made from willow compost resulted in plant deformation, which is typical of excessive nitrogen content.

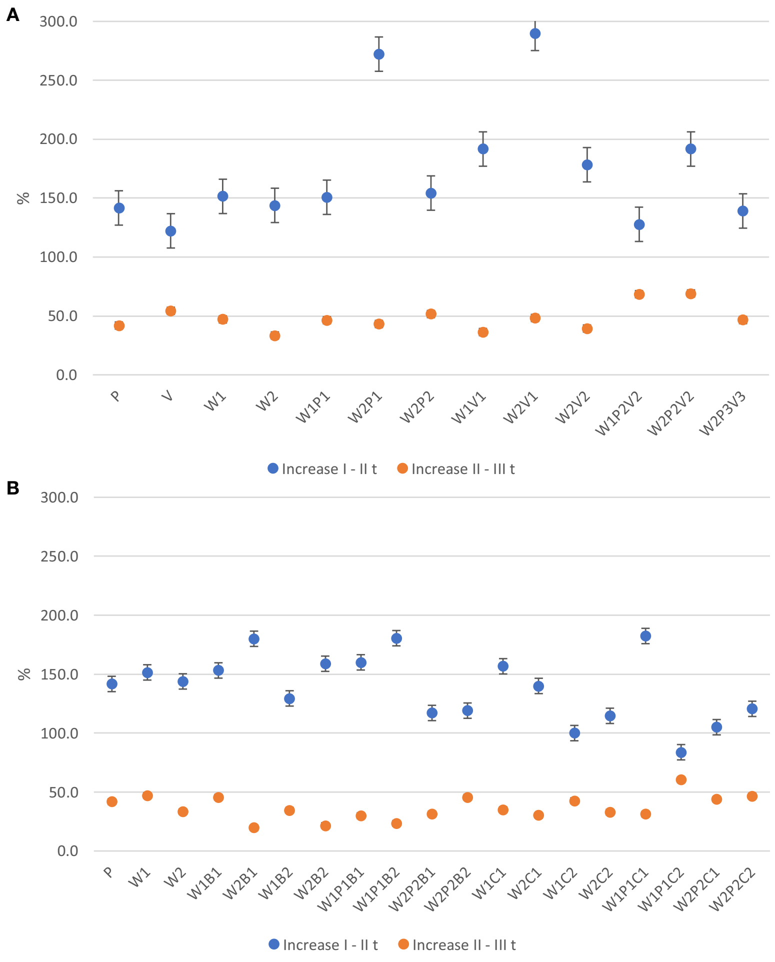

FIGURE 1
(A) The effect of horticultural media on cucumber plant height growth rate between measurement date. **(B)** The effect of horticultural media and additives on cucumber plant height growth rate between measurement date.

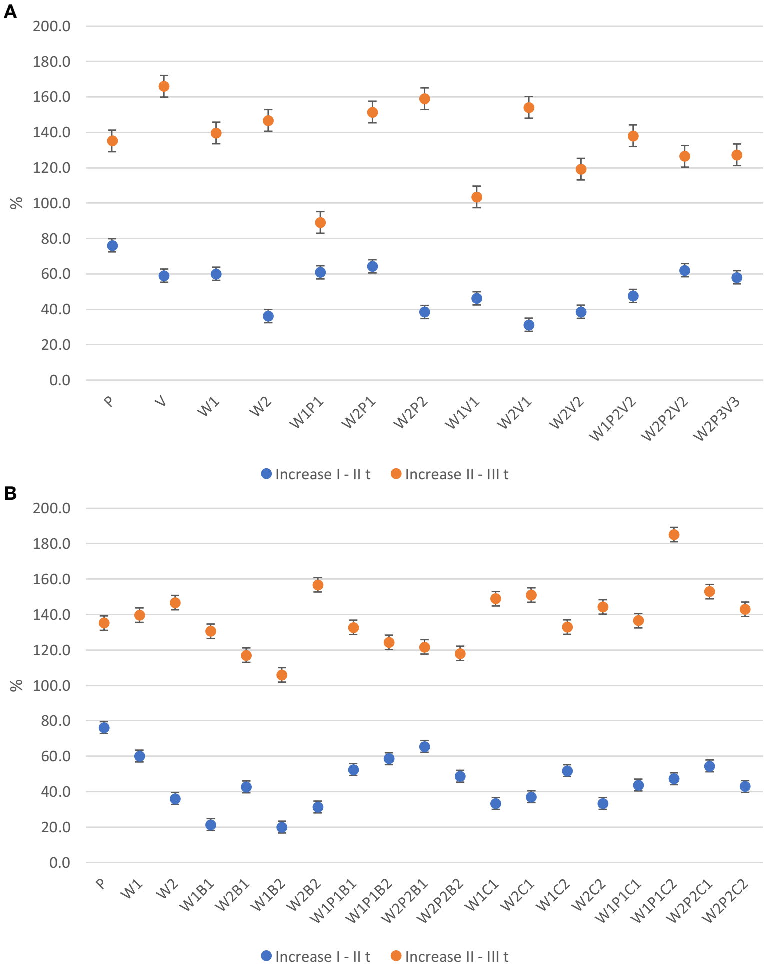

FIGURE 2
(A) The effect of horticultural media on cucumber plant lateral extent rate between measurement date. **(B)** The effect of horticultural media on cucumber plant lateral extent rate between measurement date.

However, cucumber transplants did not exhibit adverse changes in the present study. The components and additives used to prepare the growing medium influenced the plant's response, and the study demonstrated the differences in plant response.

Cucumber transplants were grown on various media, including W1P1, W2P1, W2P2, W1P1B1, W2P1B2, and W1P1C1, consisting of willow compost and 50 or 25% peat. Additionally, 5 or 10 g of basaltmeal per 100 g of substrate and 10 g of biochar were added to W1 and W2 compost mixtures. The results showed that the cucumber transplants growth was similar or even better than those grown in standard peat substrates. The quality of cucumber transplants declined when vermicompost (W1V1 and W2V2), higher amounts of basaltmeal (W1B2 and W2B2), or biochar (W1C2 and W2C2) were added to the W1 and W2 compost mixtures.

Several studies have suggested that up to 25% of peat in substrates can be replaced with different composts without negatively impacting tomato seedling quality. These findings are consistent with the results of the present study. However, the effects of vermicompost, basaltmeal, and biochar on cucumber growth were different in this study than in previous studies on different horticultural plant species. For example, Alvarez et al. (2017) recommend using 10 to 50% vermicompost as a substrate additive. Similarly, Jankauskiene et al. (2022) found that adding vermicompost to peat (up to 30%) had a beneficial effect on leaf number, cucumber plant height, and leaf area. In contrast, the present study found that the optimum proportion of vermicompost as an additive to willow composts was between 15-25%. Vermicomposting indirectly mitigates the effects of global warming and the greenhouse effect through its ability to sequester carbon media (Olle, 2019). The products obtained from vermicomposting processes depend on the feedstock used and their proportion in the mixtures. Final vermicompost product should be evaluated before utilisation considering its quality for different purposes such as organic fertilizer or substrate for seedlings production. Additionally, smaller amounts of basaltmeal (5 g per 100 g) and biochar (10 g per 100 g) as substrate additives were found to be more beneficial for cucumber growth. Basaltmeal as a rock dust is a mineral byproduct of mining, which can be used to improve the fertility of substrate, improve plant growth, enhance the activity of beneficial microflora and increase the quality of fruits and vegetables (Beerling et al., 2018). Application of basaltmeal

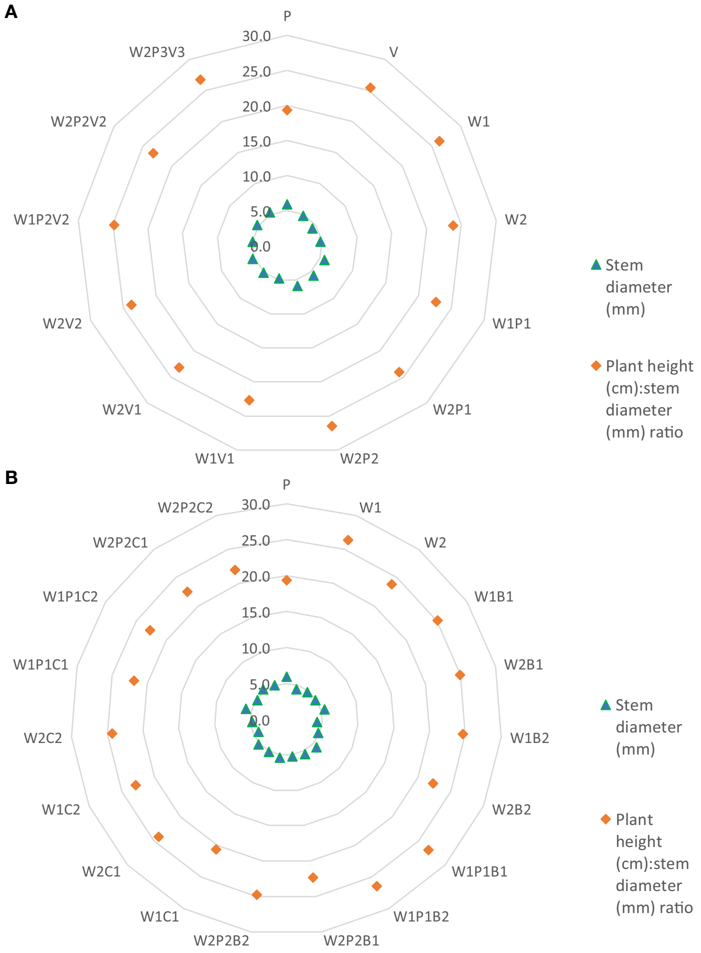

FIGURE 3
(A) The effect of horticulture media on stem diameter and plant height (cm):stem diameter (mm) ratio. **(B)** The effect of horticulture media and additives on stem diameter and plant height (cm):stem diameter (mm) ratio.

stimulated plant growth and reduced the severity of bacterial wilt in greenhouse tomato production (Li and Dong, 2013). Alvarez et al. (2017) demonstrated that 12% biochar had no negative effect on petunia growth. Biochar improves physical properties of the substrate, including surface area, density, porosity, pore size, and water-holding capacity. Biochar surface area ranges from 100 to 800 m^2/g, and porosity from 50 to 70% (Choi et al., 2019). In lettuce production (*Lactuca sativa* L.), replacing 10% of peat (in volume) by biochar, increased lettuce head mass by 184–270% compared to using peat substrate alone (Mendez et al., 2017).

A high-quality cucumber transplant is characterized by its health, developmental stage, optimal height-to-stem thickness ratio, short hypocotyl, thick green leaves and cotyledons, and well-developed roots (Yan et al., 2019; Li et al., 2013). In a recent study by Wang et al. (2021), cucumber seedlings achieved a height of 8.2-13.1 cm after 21 days of optimal cultivation, which is similar to the range of 9.7-15.5 cm observed in our experiment. However, the average plant weight of cucumber seedlings varied significantly between the two studies. In our study, it ranged from 5.3 to 10.4 g per plant, while in the study by Wang et al. (2021) it was much lower, ranging from 2.48 to 3.77 g. A more reliable indicator to assess plant growth dynamics during the initial phase is the height-to-diameter ratio (Haase, 2008; Díaz-Pérez and Camacho-Ferre, 2010; Adamczewska-Sowińska et al., 2022). In our study, the plants had a more uniform index ranging from 19.4 - for cucumber transplants produced in a peat medium (P) to 26.8 in the W1P1B1 medium.

Conclusion

Growing media composed solely of a mixture of different willow composts, combined in varying proportions, did not provide equivalent growing conditions to that of the peat substrate during the initial growth period of cucumber plants. Conversely, the study demonstrated that the W1 and W2 mixtures can partially replace peat as one of the substrate components. Cucumber transplants with comparable or greater weight than those cultivated in peat substrate were obtained when the proportion of peat in the willow compost substrate ranged between 25-50%. Obtaining transplants with comparable characteristics to those grown in peat substrate, while utilizing up to 75% less of this valuable environmental

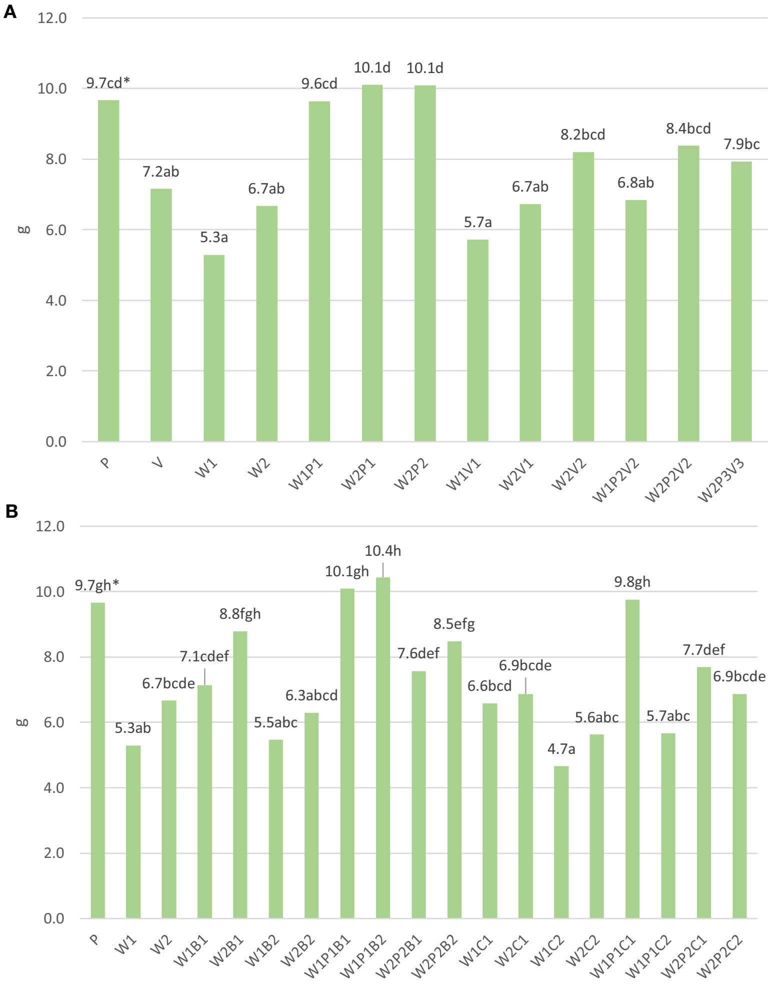

FIGURE 4
(A) The effect of horticulture media on one cucumber plant mass (g). *Means that include a common letter are not statistically significant. **(B)** The effect of horticulture media and additives on one cucumber plant mass (g). *Means include a lowercase letter are not statistically significant.

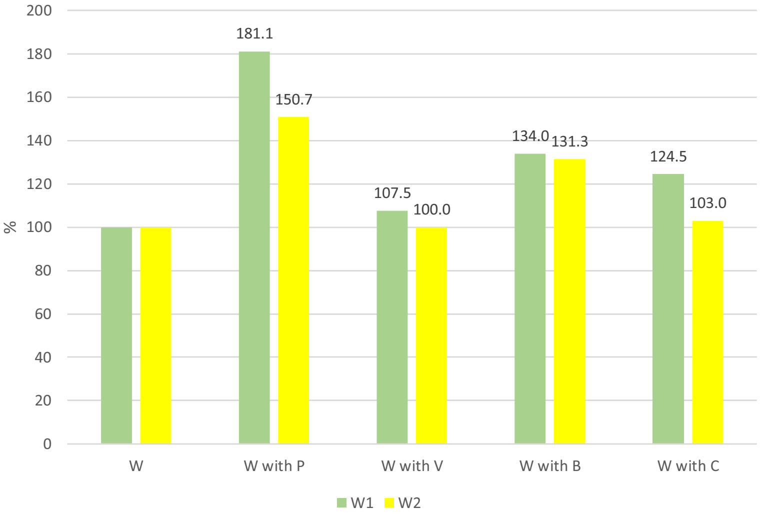

FIGURE 5
The effect of horticulture media additives on one plant mass changes (in %). The base value (100%) a cucumber plant mass on produced on compost media (W1 and W2) without media component and additives.

material, holds significant importance for the development of this type of production. Furthermore, it should be emphasized that cucumber transplants cultivated on the optimal substrates containing willow compost were healthy, did not display any signs of disease, well develop, and maintained a height-to-stem thickness ratio over 25.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

JS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. KA-S: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. EJ: Investigation, Methodology, Validation, Writing – review & editing. JB: Investigation, Methodology, Validation, Writing – review & editing.

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Conflict of interest

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

Publisher's note

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EDITED BY
Giao Nguyen,
Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia

REVIEWED BY
Guodong Liu,
University of Florida, United States
Kusumiyati Kusumiyati,
Padjadjaran University, Indonesia

*CORRESPONDENCE
Zhao Song
✉ songzhao@gdaas.cn
Faisal Nadeem
✉ fnadeem90@gmail.com;
✉ faisal.ss@pu.edu.pk

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Comparative efficacy of non-electric cooling techniques to reduce nutrient solution temperature for the sustainable cultivation of summer vegetables in open-air hydroponics

Muhammad Mohsin Nisar[1,2], Rashid Mahmood[3], Salman Tayyab[2], Moazzam Anees[2], Faisal Nadeem[1,3]*, Sadia Bibi[4], Faiza Waseem[3], Nazir Ahmed[5], Jing Li[1] and Zhao Song[1]*

[1]Key Laboratory for New Technology Research of Vegetables, Vegetable Research Institute, Guangdong Academy of Agricultural Science, Guangzhou, China, [2]Department of Horticulture, University of the Punjab, Lahore, Pakistan, [3]Department of Soil Science, University of the Punjab, Lahore, Pakistan, [4]Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan, [5]College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong, China

The cultivation of summer vegetables in open-air nutrient film technique (NFT) hydroponics is limited due to the elevated nutrient solution temperature (NST). In this regard, non-electric evaporative-cooling techniques were explored to maintain NST in open-air NFT hydroponics. Four cooling setups were employed by wrapping polyvinyl chloride (PVC) grow pipes with one and two layers of either wet or dry jute fabrics and attaching them with coiled aluminum pipe buried inside a) wet sand-filled brick tunnels (Cooling Setup I), b) two inverted and vertically stacked earthen pots (Cooling Setup II), c) two inverted and vertically stacked earthen pots externally wrapped with wet jute fabric (Wrapped Cooling Setup II), and d) an earthen pitcher wrapped with wet jute fabric (Cooling Setup III). Wrapping grow pipes with two layers of wet jute fabric reduced NST by 5°C as compared to exposed (naked) grow pipes. The double-layer jute fabric-wrapped grow pipes produced 182% more reduction in NST in comparison to single layer-wrapped grow pipes. Additionally, the installation of Wrapped Cooling Setup II and Cooling Setup III outperformed Cooling Setup I and Cooling Setup II through NST reduction of approximately 4°C in comparison to control. Interestingly, Cooling Setup III showed its effectiveness through NST reductions of 193%, 88%, and 23% during 11 a.m.–12 p.m. as compared to Cooling Setup I, Cooling Setup II, and Wrapped Cooling Setup II, respectively. In contrast, Wrapped Cooling Setup II caused NST reductions of 168%, 191%, and

18% during 2–3 p.m. in comparison to Cooling Setup I, Cooling Setup II, and Cooling Setup III, respectively. Thus, the double-layer jute fabric-wrapped grow pipes linked with Wrapped Cooling Setup II can ensure summer vegetable cultivation in open-air NFT hydroponics as indicated by the survival of five out of 12 vegetable plants till harvest by maintaining NST between 26°C and 28°C.

KEYWORDS

hydroponics, nutrient solution temperature, PVC grow pipes, jute fabric, open air system

1 Introduction

Hydroponic systems include deep water culture, ebb and flow, drip system, aeroponics, wick system, vertical farming system, and nutrient film technique (NFT) (Roberto, 2000). NFT hydroponic cultures have benefits over soil cultures such as efficient utilization of resources (water, nutrients, and space), better control of pests and diseases, faster growth, and higher yields. However, high initial cost, power dependency, system failure risks, limited crop compatibility, dependence on technical expertise, intensive monitoring, and system maintenance are the common limitations of NFT hydroponics (Swain et al., 2021). Given the rate of population increase and subsequent urbanization (Velazquez-Gonzalez et al., 2022), the food demand of the growing world population can only be met through the strengthening of alternate plant growth mediums. NFT hydroponics can ensure higher yield year-round through the accommodation of increased plant population of multiple crops by the effective utilization of its horizontal as well as vertical surface area compared to traditional farming systems. Moreover, the minimal escape of pesticides and fertilizers in NFT hydroponic cultures ensures lesser environmental contamination (van Delden et al., 2021).

The nutrient solution, either pumped from a reservoir tank or kept stagnant in aerated containers, remains in direct contact with plant roots in NFT hydroponics. Simple as well as modified NFT hydroponics systems are used for the commercial cultivation of vegetable crops, like lettuce, tomato, cucumber, herbs, and other green vegetables (Stephanie, 2018; Sela Saldinger et al., 2023). Various attributes of nutrient solution, e.g., nutrient concentration, dissolved oxygen, temperature, pH, and electrical conductivity, are of critical importance and need to be monitored regularly to ensure healthy crop production (Jones, 2004). Among these attributes, temperature is an important factor determining plant survival through root and shoot development in NFT hydroponics. Any temperature fluctuations, from an optimum range, subject plants to adverse stress through changes in nutrient solubility, uptake, and proportions of dissolved oxygen in the rhizosphere (Jones, 2004; Ylivainio and Peltovuori, 2012; Xia et al., 2021). The reduction of dissolved oxygen under high temperatures can prove drastic for plant growth and development. Vegetable plant species are reported to have differences in their desired optimum temperature. For instance, the optimum root zone temperature is reported to be 25°C for tomato (Tindall et al., 1990), whereas cucumber produces a higher yield at 22°C (Al-Rawahy et al., 2018). On an overall basis, 26.7°C (80°F) is the nutrient solution temperature where maximum absorption of nutrient elements is reported; however, 25°C (77°F) is considered to be the ideal temperature for maximum root and shoot growth (Jones, 2004). Nonetheless, the seasonal air temperature determines the extent of temperature fluctuations happening in the nutrient solution of the NFT system. In winter, maintaining the temperature at 28°C and 20°C proved optimum for the production of spinach and cucumber, respectively (Nxawe et al., 2009; Yan et al., 2013).

The cultivation of in-season summer vegetable crops in an open-air hydroponics can save the cost of house enclosure and air conditioning for temperature maintenance. However, maintaining nutrient solution temperature lower than the external air temperature is a challenge in an open-air hydroponic system. Thus, it becomes critically important to explore cooling systems for nutrient solutions in NFT hydroponics. In the view of high installation costs of NFT hydroponic systems, the effectiveness of non-electric techniques (jute fabric wrapping and aluminum coil buried in brick walls, earthen pots, and pitchers), to keep the nutrient solution temperature at an optimum range, has never been reported. The study aims to explore the non-electric techniques focusing, specifically, on passive evaporation to maintain nutrient solution temperature in NFT hydroponics for the cultivation of summer vegetables. This investigation is motivated by the desire to achieve nutrient solution temperature reductions without the association of expensive (electric energy-driven) methods.

2 Materials and methods

The NFT hydroponic setup was installed in the wire house of the Department of Soil Science, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The setup comprised a nutrient solution tank, a submerged solution circulating pump, an aeration pump, connecting tubes, and polyvinyl chloride (PVC) pipes. Each PVC pipe had 4-m length and 10-cm diameter, with 10 planting holders acting as a grow pipe (Figure 1). The nutrient solution of NFT hydroponic system consisted of 2 mM NH_4NO_3, 0.25 mM KH_2PO_4, 0.75 mM K_2SO_4, 0.1 mM KCl, 2 mM $CaCl_2$, 0.65

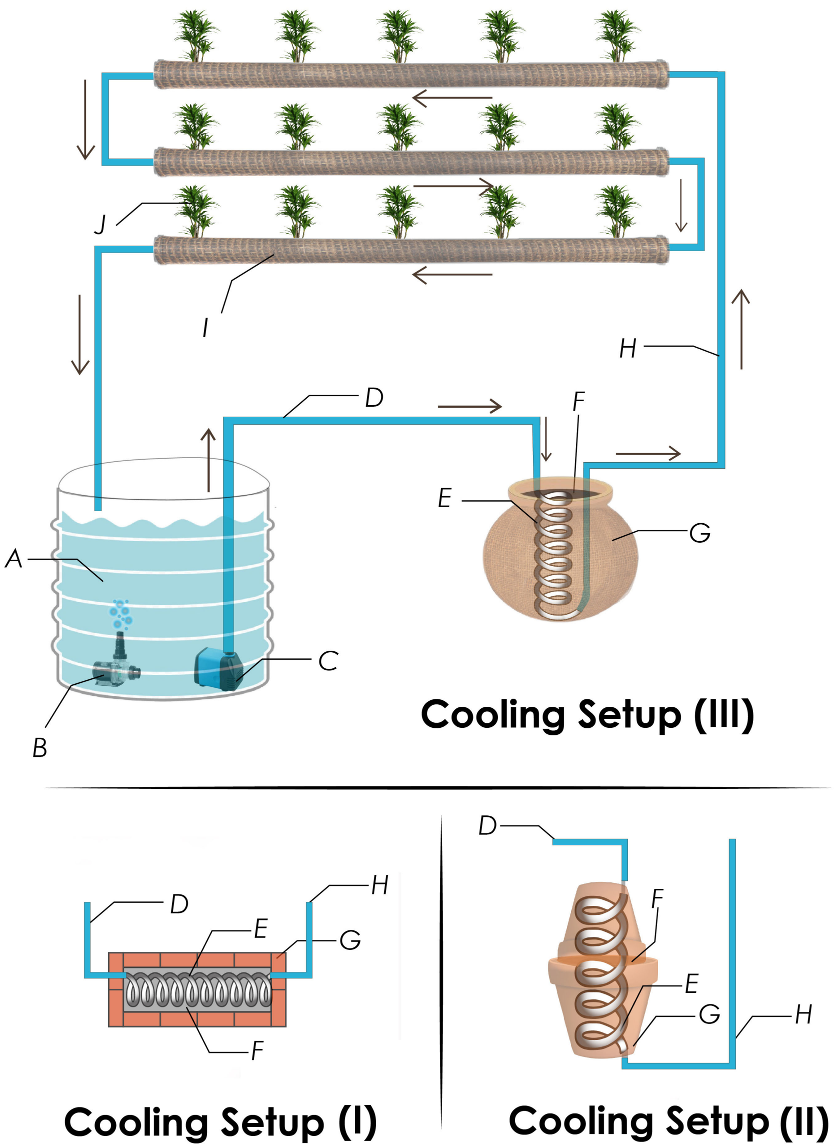

FIGURE 1
Schematic diagram of nutrient film technique (NFT) mounted with one of the three solution cooling setups. Various parts of the NFT include nutrient solution tank (A), aeration pump (B), and nutrient circulating pump (C), which pumps nutrients solution through input pipe (D) of a cooling setup to the inner aluminum pipe coil (E) that is buried in wet sand (F) to transfer heat to the external evaporative surface (G) of brick lining (Cooling Setup I), earthen pots (Cooling Setup II), or pitcher (Cooling Setup III). After that, the solution moves through output pipe (H) of a cooling setup to the jute-wrapped grow pipes (I) and ultimately back to the solution tank.

mM $MgSO_4$, 0.2 mM Fe-EDTA, 1×10^{-3} mM $MnSO_4$, 1×10^{-3} mM $ZnSO_4$, 1×10^{-4} mM $CuSO_4$, 5×10^{-6} mM $(NH_4)_6Mo_7O_{24}$, and 1×10^{-3} mM H_3BO_3 (Nadeem et al., 2018). The NFT hydroponics system was operated in open-air conditions during the summers (May 16 to July 2, 2022) under the daytime air and nutrient solution temperatures as described in Figure 2.

2.1 Experiment 1: jute fabric wrapping of grow pipes to reduce nutrient solution temperature

In this experiment, the investigations were focused on the utilization of jute fabric for wrapping PVC grow pipes as a tool to reduce nutrient solution temperature (NST). The experiment followed a completely randomized design (CRD) and considered three factors: the number of jute fabric layers used in wrapping (single-layer wrapping and double-layer wrapping), the condition of wrapped jute fabric (wet or dry), and the time of NST measurement (7 a.m.–10 a.m. and 11 a.m.–5 p.m.). The jute fabric in single-layer wrapping and double-layer wrapping was kept either dry or saturated with water once in 24 hours. The saturation proved to be effective in ensuring a continuous water supply within the fabric supporting a 24-hour evaporation cycle. The temperature variations in the nutrient solution were recorded at least twice during both designated time intervals (7 a.m.–10 a.m. and 11 a.m.–5 p.m.). These time intervals were chosen based on the

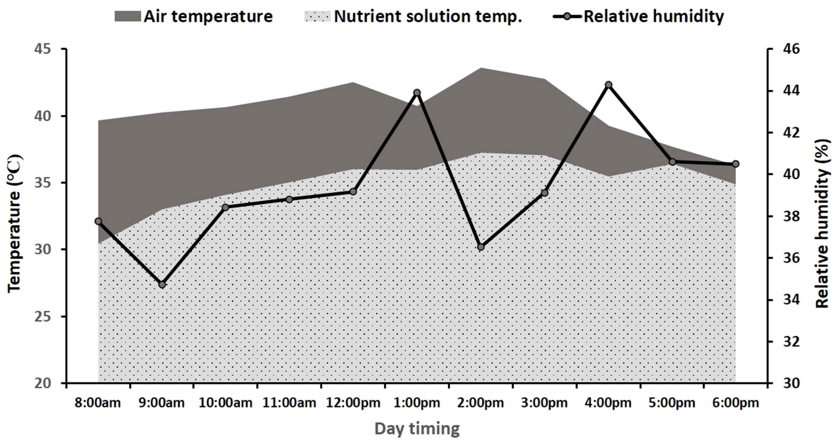

FIGURE 2
Variations with day timing in air temperature, relative humidity, and nutrient solution temperature in nutrient film technique (NFT) naked pipes (data are averages of 47 days from May 17, 2022, to July 2, 2022).

elimination of overnight cooling of the nutrient solution by approximately 10 a.m. The experiment was conducted in three replications (a 4-m-long PVC grow pipe with 10 plant holders as one replicate). Each replicate pipe was connected to a separate solution tank containing a solution circulating pump and an aeration nozzle. Cucumber seedlings at four leaf stages were transplanted to grow pipes, and the setup was run for 15 days (May 17, 2022, to May 31, 2022). During the experimental period, the wilted plants were replaced on the same day they were wilted.

Nutrient solution temperature, air temperature, and relative humidity were recorded four to five times during the day using a hygrometer (HTC-2; Walmart Canada, Mississauga, ON, Canada) equipped with an internal and cable-mounted external temperature sensor. The decrease in nutrient solution temperature in a jute fabric-wrapped grow pipe was recorded in comparison to that in a naked pipe (no wrapping) in two different time intervals (before 10 a.m. and from 11 a.m. to 5 p.m.), and the averages were calculated for each set.

2.2 Experiment 2: designing evaporative-cooling setups for the reduction of nutrient solution temperature

Four non-electric evaporative-cooling setups were developed (Figure 1) and installed in the circuit of nutrient solution individually. The details of these cooling setups are as follows.

2.2.1 Cooling Setup I (treatment 1)

This setup was built on the floor by constructing a brick tunnel of 15-cm external height, 25-cm width, 75-cm length, and 4,875 cm^2 of total external surface area for evaporation. The brick tunnel consisted of six sides in total: five sides ($15 \times 25 = 375$ cm^2, $15 \times 25 = 375$ cm^2, $15 \times 75 = 1{,}125$ cm^2, $15 \times 75 = 1{,}125$ cm^2, and $25 \times 75 = 1{,}875$ cm^2) exposed to air whereas the sixth side facing the ground and not contributing toward evaporative cooling. The total external surface area (4,875 cm^2) was calculated by summing up the areas of all five air-exposed rectangular sides. An aluminum pipe of 0.79-cm diameter and 457-cm length was shaped into a coil with 6-cm diameter and 60-cm length and attached to the nutrient solution circuit such that the nutrient solution coming from the reservoir passed through the coil before entering the grow pipes. The coil was buried in the wet sand placed inside the brick tunnel structure. The roof of the tunnel was also covered with bricks (Figure 1). The whole structure and sand were placed in a shady area and saturated with water once in 24 hours.

2.2.2 Cooling Setup II (treatment 2)

In this setup, the aluminum coil (similar to that used in Cooling Setup I) was buried in sand and placed inside two conical frustum-shaped earthen pots each of height (h) 35.0 cm, slant height (s) 37.5 cm, bottom radius (r) 4.0 cm, and top radius (R) 17.5 cm. These earthen pots were positioned such that one was inverted over the other, making the lateral surfaces of both the pots and bottom of the upper inverted pot available for evaporation (Figure 1). The evaporative surface areas of the lateral surface (LA) and bottom circular surface (BA) of the inverted pots were calculated by the following formulae (Weisstein, 2005);

$$LA = \pi \times (R + r) \times s$$

$$BA = \pi r^2$$

In this way, the total evaporative surface area (TA) of the setup was calculated to be 5,117.6 cm^2 by using the following formula:

$$TA = 2LA + BA$$

2.2.3 Wrapped Cooling Setup II (treatment 3)

Cooling Setup II was modified by wrapping the external surface of earthen pots with wet jute fabric to facilitate evaporation.

2.2.4 Cooling Setup III (treatment 4)

In this setup, the aluminum coil (similar to that used in Cooling Setup I) was immersed in the sand within a spherical earthen pitcher having a radius of 19.2 cm and a total surface area of 4,630.1 cm^2. The evaporative surface area was calculated as 4,581.4 cm^2 by subtracting the area of the circular mouth opening (28.3 cm^2) and that of the ground touching the circular base (20.4 cm^2) from the total surface area. The mouth opening of the pitcher was used for the entry and exit of the nutrient solution pipes. Therefore, the small amount of evaporation from this area was ignored.

All four cooling setups, including sand, were placed in a shaded area and saturated with water once in 24 hours. Through initial investigations, it was found that the periodic saturation effectively maintains sufficient water for evaporation throughout the structure until the subsequent saturation cycle. The NFT setup having none of the aforementioned cooling setups was taken as control. Each of the four cooling setups was attached with three grow pipes containing cucumber plants in holders and ran for 15 days from June 3, 2022, to June 18, 2022, in triplicate. Nutrient solution temperature, external air temperature, and relative humidity were recorded daily at four different time intervals, i.e., 8–9 a.m., 11 a.m.–12 p.m., 2–3 p.m., and 4–5 p.m., using a hygrometer as discussed in the case of experiment 1.

The experiment was conducted following the CRD with the type of cooling setups (Cooling Setup I, Cooling Setup II, Wrapped Cooling Setup II, and Cooling Setup III) and time intervals of nutrient solution temperature recording (8–9 a.m., 11 a.m.–12 p.m., 2–3 p.m., and 4–5 p.m.) as experimental factors. At a particular interval, the decrease in nutrient solution temperature due to a cooling setup was recorded in comparison to the control. The average values of 15 days were used in the statistical analysis.

2.3 Testing of the selected cooling techniques for the cultivation of summer vegetables

Keeping in view the results of experiment 1 and experiment 2, Wrapped Cooling Setup II was connected to double-layer wet jute fabric-wrapped grow pipes in the NFT system and tested for the cultivation of brinjal (*Solanum melongena*), okra (*Abelmoschus esculentus*), spinach (*Spinacia oleracea*), bitter gourd (*Momordica charantia*), coriander (*Coriandrum sativum*), sponge gourd (*Luffa aegyptiaca*), bottle gourd (*Lagenaria siceraria*), summer squash (*Cucurbita pepo*), pumpkin (*Cucurbita moschata*), cucumber (*Cucumis sativus*), and snake gourd (*Trichosanthes cucumerina*). An NFT system with naked grow pipes but none of the cooling setups in the circuit was considered as control. Three seedlings of each vegetable (Table 1), at four leaf stages, were shifted to grow pipes, and their survival duration was recorded for up to 2 months. The survival duration of the seedlings was recorded as the time taken by the seedlings from transplanting to permanent wilting. The confirmation of permanent wilting was performed by observing the plants in the grow holders for the subsequent 3 days. Two months after transplanting, the survived vegetable seedlings were carefully removed from grow holders and assessed for their growth in terms of root length, shoot length, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. The number of leaves was counted, and the root and shoot lengths were measured using a meter rod. The shoot was separated from the root using a scissor and weighed through digital balance for shoot fresh weight. Any nutrient solution, adhering to the roots, was gently blotted with tissue paper for the measurement of root fresh weight. The dry weight was determined by keeping the shoot and root samples in an oven at 60°C till constant weight. For all the recorded growth parameters, the mean of the three seedlings of each vegetable was calculated and reported in Table 2. During the first week of the experiment, the temperature variations in the nutrient solutions were monitored at approximately 3 p.m. daily.

TABLE 1 Survival time of various vegetable seedlings cultivated in naked grow pipes and grow pipes wrapped with two layers of wet jute fabric and attached with wrapped Cooling Setup II.

Vegetables cultivated	Plant survival time	
Common name	Naked grow pipes	Wrapped grow pipes and wrapped Cooling Setup II in circuit
Brinjal (*Solanum melongena*)	7 days	Survived till harvest
Okra (*Abelmoschus esculentus*)	6 days	26 days
Spinach (*Spinacia oleracea*)	3 days	3 days
Bitter gourd (*Momordica charantia*)	7 days	Survived till harvest
Coriander (*Coriandrum sativum*)	2 days	2 days
Sponge gourd (*Luffa aegyptiaca*)	6 days	Survived till harvest
Bottle gourd (*Lagenaria siceraria*)	7 days	Survived till harvest
Summer squash (*Cucurbita pepo*)	3 days	4 days
Pumpkin (*Cucurbita moschata*)	4 days	6 days
Cucumber (*Cucumis sativus*)	6 days	Survived till harvest
Snake gourd (*Trichosanthes cucumerina*)	5 days	15 days

Survival time is an average of at least three plants of a species.

2.4 Statistical analysis

For experiment 1, involving nutrient solution temperature reduction through wrapping of PVC grow pipes, the analysis of variance (ANOVA) was performed using Statistix (v. 8.1) to separate the main effects of three factors (number of jute layers

TABLE 2 Growth of vegetable seedlings survived till harvest in grow pipes wrapped with two layers of wet jute fabric and attached with wrapped Cooling Setup II.

Parameter	Brinjal	Bitter gourd	Sponge gourd	Bottle gourd	Cucumber
Root length (cm)	15.2 ± 1.4	10.2 ± 1.4	133.1 ± 12.05	7.2 ± 1.1	5.8 ± 0.6
Shoot length (cm)	36.6 ± 2.2	259.9 ± 13.5	995.7 ± 41.3	155.4 ± 2.9	75 ± 2.7
No. of leaves	8.4 ± 2.3	40.1 ± 4.2	56.7 ± 45.1	28.6 ± 13.5	25.6 ± 9.3
Shoot fresh weight (g)	4.9 ± 0.3	17.6 ± 3.9	138.7 ± 15.6	12.6 ± 3.3	13.3 ± 8.5
Root fresh weight (g)	0.5 ± 0.1	2.3 ± 0.3	32.7 ± 3.7	1.6 ± 3.7	2.2 ± 0.7
Shoot dry weight (g)	2.1 ± 0.2	5.6 ± 0.2	52.0 ± 2.7	3.1 ± 0.4	1.9 ± 0.1
Root dry weight (g)	0.1 ± 0.0	0.2 ± 0.0	3 ± 0.2	0.9 ± 0.1	0.1 ± 0.03

Values are mean ± SE.

wrapped, dry versus wet jute, and time of observation) as well as their interactions (Table 3). In addition, ANOVA was performed to find the effect of rates within each factor. The mean comparison of nutrient solution temperature reductions in wrapped grow pipes with respect to naked grow pipes was performed using Tukey's honestly significant difference (HSD) test. For the comparison of four cooling systems in experiment 2, ANOVA was performed using Statistix (v. 8.1) to separate the main effects of factors (cooling system types and time of observation) as well as their interactions. In addition, ANOVA was also performed to find the effect of cooling systems on the nutrient solution temperature measured at different times of the day. The mean comparison of nutrient solution temperature reductions in grow pipes attached cooling setup with respect to control pipes (no cooling setup attachment) was made using Tukey's HSD test.

3 Results

Initially, the high nutrient solution temperature (up to 40°C) caused the death of all summer vegetable seedlings (Table 1) within 1 week (May 2022) of their transplantation in the NFT system. Various non-electric cooling techniques were implied and compared for their efficiency in reducing the nutrient solution temperature. During the study period, the external air temperature fluctuated from 30°C to 46°C (Figure 2). It caused the nutrient solution temperature to vary from 32°C to 37°C in naked grow pipes from 8 a.m. to 6 p.m. This range of nutrient solution temperature was 4.6°C, 3.3°C, 3.2°C, and 1.2°C lesser than the external air temperature recorded before 9 a.m., from 9 p.m. to 12 p.m., from 12 p.m. to 3 p.m., and 3 p.m. to 6 p.m., respectively. The nutrient solution temperature was minimal before 9 a.m. which increased by 3°C and 4.7°C up to 12 p.m. and 3 p.m., respectively, in naked grow pipes. However, the change in nutrient solution temperature from 3 p.m. to 6 p.m. was negligible (Figure 2). The relative humidity varied from 25% to 80% during the study period. It remained at approximately 30% to 40% from the start of May to the middle of June but fluctuated between 60% and 80% after June 15 due to pre-monsoon rains. However, the average daily relative humidity level during the study period was 35% to 44% (Figure 2).

TABLE 3 Analysis of variance for the impact of wrapping of nutrient solution carrying polyvinyl chloride (PVC) pipes with jute cloth on the solution temperature in nutrient film technique (NFT).

Source	F-ratio	p-Value
No. of jute layers wrapped (NJL)	11.59	0.001*
Dry vs. wet jute (DWJ)	75.30	0.000*
Time of observation (t)	16.13	0.000*
NJL × DWJ	0.47	0.497
DWJ × t	0.10	0.752
NJL × t	0.02	0.881
JW × t × NJL	0.48	0.489

*Significant at $p \leq 0.001$. ns, non-significant at $p< 0.05$.

3.1 Impact of jute fabric wrapping of grow pipes on nutrient solution temperature

The results of ANOVA highlighted the significant influence of grow pipe wrapping techniques to reduce nutrient solution temperature at different times of observations (Table 3). However, the interaction effects of the number of jute fabric layers wrapped, dry or wet jute fabric layer, and time of observation were non-significant at p<0.05 (Table 3). The nutrient solution temperature in jute fabric-wrapped grow pipes decreased by up to 5°C in comparison to naked grow pipes. Furthermore, the double-layer jute fabric wrapping proved approximately 20% more effective in reducing the solution temperature than the single-layer wrapping (Figure 3A, Supplementary Table 1). Consistently, the wet jute fabric wrapping proved to decrease nutrient solution temperature by 182% more than dry jute fabric wrapping (Figure 3B, Supplementary Table 1). On average, the decrease in nutrient solution temperature under double-layer jute fabric wrapping was 74% more than single-layer wrapping at all times of observation (Supplementary Table 1); however, the decrease was 39% more prominent during 11 a.m.–5 p.m. than 7 a.m.–10 a.m. (Figure 3C).

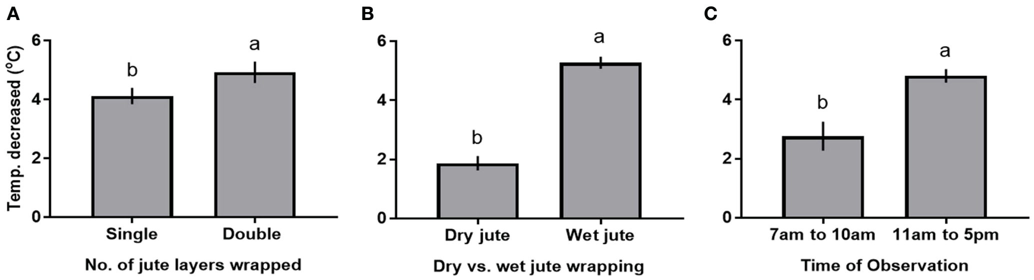

FIGURE 3
Main effects of number of jute layers wrapped over nutrient film technique (NFT) solution pipes **(A)**, dryness or wetness of the wrapped jute **(B)**, and time of the day **(C)** on the decrease in solution temperature in comparison to that in the naked pipe. Bars are means ± standard errors, and means sharing common letters do not differ significantly at $p < 0.05$.

3.2 Impact of installation of various non-electric cooling setups on nutrient solution temperature

The evaluation of the impact of cooling setups on nutrient solution temperature ($p < 0.05$) indicated decreases in nutrient solution temperature under all four cooling setups (Figure 4). The results demonstrated statistically similar decreases in nutrient solution temperature at all times of observations under Cooling Setup I and Cooling Setup II installments. A maximum of 1.8°C decrease in nutrient solution temperature was observed under Cooling Setup I and Cooling Setup II installments in comparison to control (no cooling setup installation). Wrapped Cooling Setup II and Cooling Setup III resulted in statistically similar reductions in solution temperature as compared to Cooling Setup I and Cooling Setup II from 8 a.m. to 9 a.m. (Figure 4). However, the temperature decreases under Wrapped Cooling Setup II were 168% and 191% higher than Cooling Setup I and Cooling Setup II, respectively, from 2 p.m. to 3 p.m. (Supplementary Table 2). To be precise, the temperature decreases due to Wrapped Cooling Setup II ranged from 3.6°C to 4.5°C and recorded as maximum from 2 p.m to 3 p.m. Cooling Setup III worked in a sustainable way from 11 a.m. to 5 p.m. and decreased nutrient solution temperature by approximately 3.5°C in comparison to control (Figure 4). Overall, the performances of Wrapped Cooling Setup II and Cooling Setup III to decrease the nutrient solution temperature were, statistically, alike (Figure 4).

3.3 Survival of summer vegetables in NFT hydroponic system having double-layer wet jute fabric-wrapped grow pipes attached with Wrapped Cooling Setup II

The double-layer wet jute fabric-wrapped grow pipes attached with Wrapped Cooling Setup II reduced nutrient solution temperature by 3°C–8°C in comparison to control, i.e., naked grow pipes without any cooling setup installation (Figure 5). The temperature fluctuations in the nutrient solution of control remained between 30°C and 37°C, which resulted in vegetable

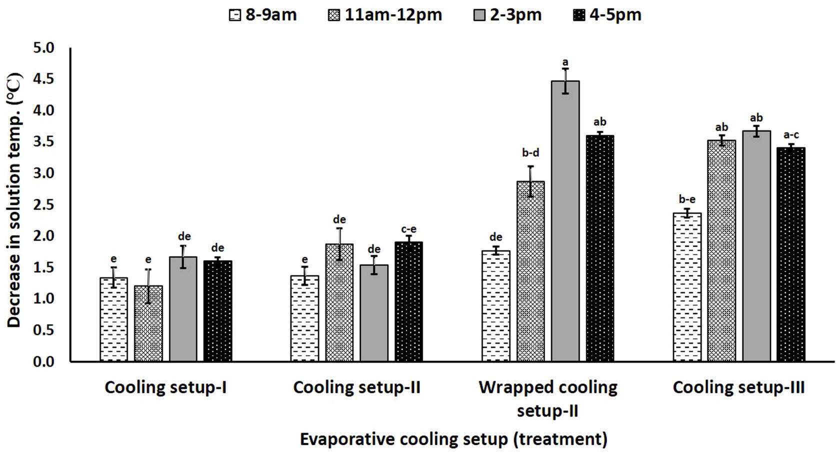

FIGURE 4
Impact of various non-electric evaporation techniques on decrease in nutrient solution temperature in nutrient film technique. Bars are means ± standard errors, and means sharing common letters do not differ significantly at $p < 0.05$.

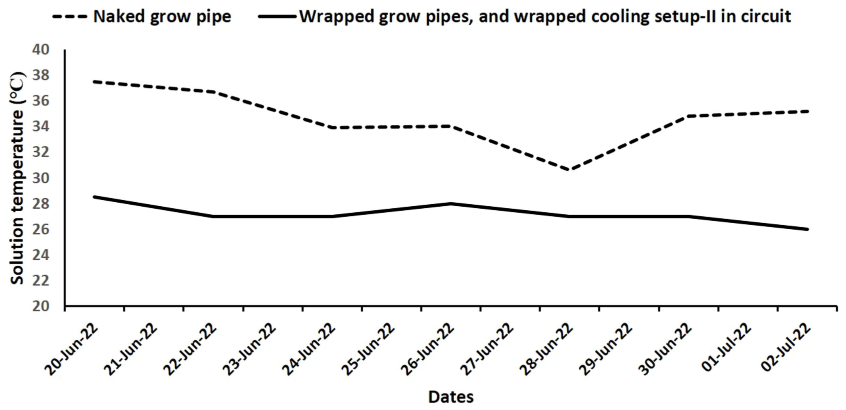

FIGURE 5
Comparison of nutrient solution temperature in naked grow pipes and wrapped grow pipes attached to wrapped Cooling Setup II during the first 2 weeks of test cultivation of vegetable crops.

seedling death within a week of the study period (Table 1). However, five out of 11 vegetable plants, viz., brinjal (*S. melongena*), bitter gourd (*M. charantia*), sponge gourd (*L. aegyptiaca*), bottle gourd (*L. siceraria*), and cucumber (*C. sativus*), survived in the double-layer wet jute fabric-wrapped grow pipes attached to Wrapped Cooling Setup II (Tables 2, 3). Nonetheless, the wilting of the other seven vegetable plants took 2–26 days, a greater survival time in comparison to that noted in naked grow pipes (Table 1).

4 Discussion

An evaluation of very unique non-electric cooling techniques was carried out for their effectiveness in reducing the nutrient solution temperature of an NFT hydroponics system for the cultivation of summer vegetables. The nutrient solution temperature maintenance enables plant roots to uptake nutrients and water to ensure optimum growth and development (Solfjeld and Johnsen, 2006; Yan et al., 2012). In this study, the initial death of vegetable seedlings in naked grow pipes was the result of increased temperature (40°C) in nutrient solution. Summer vegetable canopies are supposed to withstand high temperatures, but their roots need to reside at below 30°C (86°F), which is maintained in the soil system (Suzuki, 1966). Thus, a temperature value higher than the optimum level would have seized the physico-chemical processes in roots, leading to the impairment of photosynthetic activity owing to a lack of sufficient water and nutrient supply (Xia et al., 2021). The minimum exposure to the external environment, through jute fabric wrapping of grow pipes, reduced the nutrient solution temperature and improved the photosynthetic framework of leaves. Given the differences in heat intensities of the external environment (Hooks et al., 2022), the decrease in nutrient solution temperature proved more effective during 11 a.m.–5 p.m. than that before 10 a.m. In addition to the reduced exposure to the external environment, these fluctuations in external temperature intensities were better regulated through wet jute fabric wrapping than dry jute fabric wrapping of grow pipes through the evaporative-cooling effect caused by the escape of high energy liquid molecules (Wang et al., 2017).

Given the principle of the “evaporative-cooling effect”, the installation of cooling setups with jute fabric-wrapped grow pipes proved successful in decreasing nutrient solution temperature. The various components of cooling setups, viz., porous walls of the bricks, earthen pot, and pitcher, allowed high-energy water molecules to escape the wet sand and evaporate. The attachment of double-layer wet jute fabric-wrapped grow pipes with wrapped Cooling Setup II and Cooling Setup III increased the effectiveness of the cooling systems in reducing nutrient solution temperature. We argued that the evaporative cooling of sand lowered the temperature of nutrient solution circulating in sand-buried aluminum coil in addition to the temperature decrease resulting from the evaporative cooling through double-layer wet jute fabric (Yang et al., 2019). Moreover, the wet jute fabric kept the evaporative surfaces wet and helped in establishing a continuity of evaporative streams, which, otherwise, might have broken due to the dryness of grow pipes, pitcher, or earthen pot surfaces. Additionally, more than evaporative cooling, the sunlight-exposed naked grow pipes experienced radiation energy-based direct heating (Guo et al., 2020). The absence of evaporative cooling-based temperature reductions made Cooling Setup I and Cooling Setup II less effective than Wrapped Cooling Setup II and Cooling Setup III.

The double-layer wet jute fabric-wrapped grow pipes attached to Wrapped Cooling Setup II maintained the nutrient solution temperature at 26°C–28°C, which was comparable to the recommended temperature of 26°C for hydroponics (González-García et al., 2023). This maintenance of nutrient solution temperature allowed five vegetable species (brinjal, bitter gourd, sponge gourd, bottle gourd, and cucumber) to survive till harvest. The other six vegetable species showed variable survival duration. This could be described as the plant-specific response to oxygen levels in the root zone. Plant species vary in their tolerance to suffocation under submergence or semi-submergence (Pradhan and Mohanty, 2013). Furthermore, the same nutrient solution recipe used for all vegetable species might have caused differential growth regulations in survived as well as wilted vegetable species. It was evident from the results of the experiment that the cooling setups never reduced nutrient solution temperature below air temperature during the morning time of respective days. Furthermore, a setup-induced decrease in temperature was less or negligible in the morning than that at noon or afternoon. These are the limitations of non-electric cooling setups studied, which provide future direction to explore the underlying reasons.

5 Conclusion

High temperature in nutrient solution is the major constraint for the survival of summer vegetable seedlings in an open-air NFT hydroponics system. The experimental results suggested that wrapping grow pipes with two layers of wet jute fabric can effectively reduce nutrient solution temperature through the evaporative-cooling effect. This effect can be further enhanced by introducing a non-electric cooling setup (Wrapped Cooling Setup II), which can keep the nutrient solution temperature within the plant tolerable range (26°C–28°C). The increased survival time of summer vegetables particularly brinjal (*S. melongena*), bitter gourd (*M. charantia*), sponge gourd (*L. aegyptiaca*), bottle gourd (*L. siceraria*), and cucumber (*C. sativus*) indicated that this method can be used to overcome the major constraint of high temperature in nutrient solution for the survival of summer vegetable seedlings in an open-air NFT hydroponics system. This method can be useful for farmers who want to grow summer vegetables in hot and dry climates.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

MM: Data curation, Investigation, Writing – original draft. RM: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. ST: Data curation, Investigation, Writing – review & editing. MA: Data curation, Formal Analysis, Writing – review & editing. FN: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. SB: Writing – review & editing. FW: Writing – review & editing. NA: Writing – review & editing. JL: Writing – review & editing. ZS: Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1340641/full#supplementary-material

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*CORRESPONDENCE
Zhao Song
songzhao@gdaas.cn
Faisal Nadeem
fnadeem90@gmail.com;
faisal.ss@pu.edu.pk

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Corrigendum: Comparative efficacy of non-electric cooling techniques to reduce nutrient solution temperature for the sustainable cultivation of summer vegetables in open-air hydroponics

Muhammad Mohsin Nisar[1,2], Rashid Mahmood[3], Salman Tayyab[2], Moazzam Anees[2], Faisal Nadeem[1,3*], Sadia Bibi[4], Faiza Waseem[3], Nazir Ahmed[5], Jing Li[1] and Zhao Song[1*]

[1]Key Laboratory for New Technology Research of Vegetables, Vegetable Research Institute, Guangdong Academy of Agricultural Science, Guangzhou, China, [2]Department of Horticulture, University of the Punjab, Lahore, Pakistan, [3]Department of Soil Science, University of the Punjab, Lahore, Pakistan, [4]Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan, [5]College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong, China

KEYWORDS

hydroponics, nutrient solution temperature, PVC grow pipes, jute fabric, open air system

A Corrigendum on

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Additional Affiliation(s)

In the published article, there was an error regarding the affiliation(s) for “Muhammad Mohsin Nisar” and “Faisal Nadeem”. As well as having affiliation(s) “2” and “3” respectively they should also have “[1]Key Laboratory for New Technology Research of Vegetables, Vegetable Research Institute, Guangdong Academy of Agricultural Science, Guangzhou, China” as affiliation “1”.

As per above changes, the numbering of all author affiliations will be changed as mentioned above (correct version).

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Incorrect Author Name

In the published article, an author name was incorrectly written as “Song Zhao”. The correct spelling is “Zhao Song”.

The initials used in the Author contributions section should be “ZS” instead of “SZ”.

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EDITED BY
Giao Nguyen,
Department of Primary Industries and Regional Development of Western Australia (DPIRD), Australia

REVIEWED BY
Mariola Staniak,
Institute of Soil Science and Plant Cultivation, Poland
Alfonso Gago Calderón,
University of Malaga, Spain
Yanxia Zhang,
Nanjing Normal University, China

*CORRESPONDENCE
Panfeng Tu
✉ tupanfeng@163.com

†These authors have contributed equally to this work

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Advancing horizons in vegetable cultivation: a journey from ageold practices to high-tech greenhouse cultivation—a review

Nazir Ahmed[1†], Baige Zhang[2†], Lansheng Deng[3], Bilquees Bozdar[4], Juan Li[1], Sadaruddin Chachar[1], Zaid Chachar[5], Itrat Jahan[4], Afifa Talpur[4], Muhammad Saleem Gishkori[4], Faisal Hayat[1] and Panfeng Tu[1*]

[1]College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong, China, [2]Key Laboratory for New Technology Research of Vegetables, Vegetable Research Institute, Guangdong Academy of Agricultural Science, Guangzhou, China, [3]College of Natural Resources and Environment, South China Agricultural University, Guangzhou, China, [4]Faculty of Crop Production, Sindh Agriculture University, Tandojam, Pakistan, [5]College of Agriculture and Biology, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong, China

Vegetable cultivation stands as a pivotal element in the agricultural transformation illustrating a complex interplay between technological advancements, evolving environmental perspectives, and the growing global demand for food. This comprehensive review delves into the broad spectrum of developments in modern vegetable cultivation practices. Rooted in historical traditions, our exploration commences with conventional cultivation methods and traces the progression toward contemporary practices emphasizing the critical shifts that have refined techniques and outcomes. A significant focus is placed on the evolution of seed selection and quality assessment methods underlining the growing importance of seed treatments in enhancing both germination and plant growth. Transitioning from seeds to the soil, we investigate the transformative journey from traditional soil-based cultivation to the adoption of soilless cultures and the utilization of sustainable substrates like biochar and coir. The review also examines modern environmental controls highlighting the use of advanced greenhouse technologies and artificial intelligence in optimizing plant growth conditions. We underscore the increasing sophistication in water management strategies from advanced irrigation systems to intelligent moisture sensing. Additionally, this paper discusses the intricate aspects of precision fertilization, integrated pest management, and the expanding influence of plant growth regulators in vegetable cultivation. A special segment is dedicated to technological innovations, such as the integration of drones, robots, and state-of-the-art digital monitoring systems, in the cultivation process. While acknowledging these advancements, the review also realistically addresses the challenges and economic considerations involved in adopting cutting-edge technologies. In summary, this review not only provides a comprehensive guide to the current state of vegetable cultivation but also serves as a forward-looking reference emphasizing the critical role of continuous research and the anticipation of future developments in this field.

KEYWORDS

seed treatments, soilless cultures, greenhouse technologies, precision agriculture, integrated pest management, digital monitoring

1 Introduction

Advancing horizons in vegetable cultivation signifies a critical evolution from traditional, soil-based methods to innovative high-tech greenhouse cultivation. This shift, pivotal in agricultural methodologies, represents a fusion of sophisticated technologies, enhanced understanding of plant biology, and a commitment to environmental stewardship and sustainable practices. Central to ensuring global food security, this transformation in agriculture begins at the nursery, a crucial stage where the life cycle of young plants determines their future health, vigor, and yield (Mohanta, 2020). Historically rooted in empirical knowledge and tailored to local environmental conditions and cultural traditions, vegetable cultivation has now embraced a comprehensive approach involving meticulous seed selection, soil preparation, and strategic management of environmental factors and pests (Thomas et al., 2019). Facing increasing food demands from a growing global population and variable climatic patterns, the need to refine vegetable cultivation practices has become more urgent than ever. This journey, extending from seedling to harvest, transcends mere growth optimization; it is about developing resilient, sustainable, and efficient agricultural systems that can adapt to contemporary challenges (Yadav et al., 2018; Zarbà et al., 2019). Propelled by technological advancements, groundbreaking plant science research, and interdisciplinary insights, this evolution is revolutionizing vegetable cultivation techniques ushering in an era marked by precision and sustainability in agricultural practices (Dhen et al., 2019; Waiba et al., 2020).

The transition from a seed to a seedling represents a vulnerable and critical phase in the life cycle of a plant. The quality of seedlings, delineated by traits, such as vigor, root development, physiological health, and resistance to diseases and pests, has a profound influence on the productivity and health of vegetable crops (Gupta and Kumar, 2020; Sarraf et al., 2020; Zulfiqar, 2021). Advanced techniques in vegetable cultivation cater to optimizing these quality metrics. For instance, precision in watering enabled by advanced irrigation systems ensures neither overwatering nor underwatering, both of which can jeopardize young plants (Warner et al., 2018). Enhanced growth media, whether organic or soilless, can fortify root development and structure, ensuring efficient water and nutrient absorption, thereby promoting seedling health and vigor (Warner et al., 2018; Mariotti et al., 2020). Furthermore, the introduction of modern methods for detecting and managing pests and diseases can substantially reduce seedling mortality. Early detection, facilitated by imaging and diagnostic tools alongside the utilization of integrated pest management (IPM) strategies, can shield seedlings from potentially debilitating infestations (Ofuya et al., 2023). Additionally, as we grapple with the multifaceted challenges posed by climate change, dwindling arable land, and heightened pest resistance, there is a compelling and urgent need to explore and adopt innovative vegetable farming techniques. By nurturing resilient crops and minimizing resource wastage, advanced farming techniques can lay the foundation for a successful, sustainable, and productive cultivation cycle (Waiba et al., 2020; Ranganath et al., 2023).

This review offers a detailed insight into contemporary advancements in vegetable cultivation practices. As the agricultural sector evolves, driven by technology and increasing food demand, understanding these shifts is vital. This study will benefit farmers, researchers, and practitioners, aiming to streamline their practices. The historical context unveils the evolution of vegetable farming from traditional to modern techniques. Emphasis on seed quality, selection, and treatment underscores their role in optimal germination. The examination of soil and growing media touches upon innovations, such as soilless cultures and coir. Advanced greenhouse technologies, artificial intelligence (AI), and automation have revolutionized environmental control, whereas water management now emphasizes modern irrigation and moisture-sensing techniques. Nutritional management focuses on precision fertilization and organic nutrients, and pest control highlights IPM strategies and biological controls. This review covers the significance of plant growth regulators and stresses in modern transplanting methods and seedling acclimatization. Technological advancements, such as drones, AI, and imaging techniques, have been highlighted, along with the importance of sustainable vegetable farming practices. Finally, the review concludes by outlining future prospects and emphasizing the central role of research in steering the industry's trajectory. This review serves as a concise guide capturing the essence of modern vegetable farming and its future direction.

2 Historical overview

2.1 Traditional methods of vegetable cultivation practices

Traditional methods in vegetable cultivation practices are deeply rooted in time-tested agricultural practices and sculpted meticulously by local environmental nuances and age-old cultural traditions. The emphasis on selecting seeds from the most robust plants underscores the primitive understanding of genetics (Figure 1). These plants, which are resilient to diseases and pests, are known to consistently yield superior produce (Reed et al., 2022). Seed treatments in this era were straightforward, yet effective. Common practices, such as soaking seeds in water or sun drying, aimed to enhance germination rates, a phenomenon detailed in the literature, such as that by Corbineau et al. (2023). In terms of soil preparation, this was an intensive hands-on affair. The ground was laboriously turned, weeds were removed, and any larger clumps were broken down to foster a nurturing environment for burgeoning seedlings. Organic matter, which predominantly decomposed farmyard manure, was meticulously mixed with the soil.

In regions susceptible to waterlogging, the landscape is often punctuated with raised beds, a strategy highlighted by Ayyam et al. (2019). Sowing is a meticulous process. Seeds were either broadcast by hand or sown in meticulously arranged lines, with each vegetable species dictating spacing to mitigate overcrowding and ensure robust growth. Traditional irrigation is, in essence, an exercise of simplicity and observation. The trade tools were rudimentary watering cans or basic hosepipes, and the cadence of watering was gauged by the keen eyes of farmers who watched for cues in soil moisture and crop vitality. Regions facing the challenge of intense sunlight or blustery winds witnessed innovation in the guise of temporary protective structures. Natural materials, such as straw or palm fronds, as referenced by Narvaez (2020), were repurposed to offer shade or counteract wind safeguarding vulnerable plants.

The solutions were predominantly organic when combatting pests and disease. Botanical remedies, such as revered neem oil or fiery garlic, chili, onion, and various other bio-pesticidal plant extract concoctions, are preferred (Baidoo and Mochiah, 2016; Benelli et al., 2017). Strategic agricultural choices, such as cultural methods, physical and mechanical barriers, sex pheromones, bio-pesticides and bio-control agents, and chemical and botanical means, have been employed as preemptive strikes against pest invasions, a sustainable approach explored in depth by Abhishek and Dwivedi (2021). The final rite of passage for seedlings, transplantation, was a meticulous endeavor. Upon reaching maturity, seedlings were gently uprooted and rehomed to the main fields, a process that demands finesse to ensure root and plant integrity. While these labor-intensive, indigenous knowledge-driven methodologies stood the test of time in their era, a relentless march of progress accompanied an era of modern agricultural innovations. This transition saw many traditional practices enhanced or replaced by mechanized and scientifically backed methods (Zhao et al., 2020). The ultimate goal of achieving unparalleled efficiency, consistency, and yields is to set the stage for the contemporary agricultural marvels we hold.

2.2 Evolution of techniques in vegetable cultivation practices over the decades

Over the decades, the evolution of vegetable cultivation techniques has offered a fascinating glimpse into the broader arc

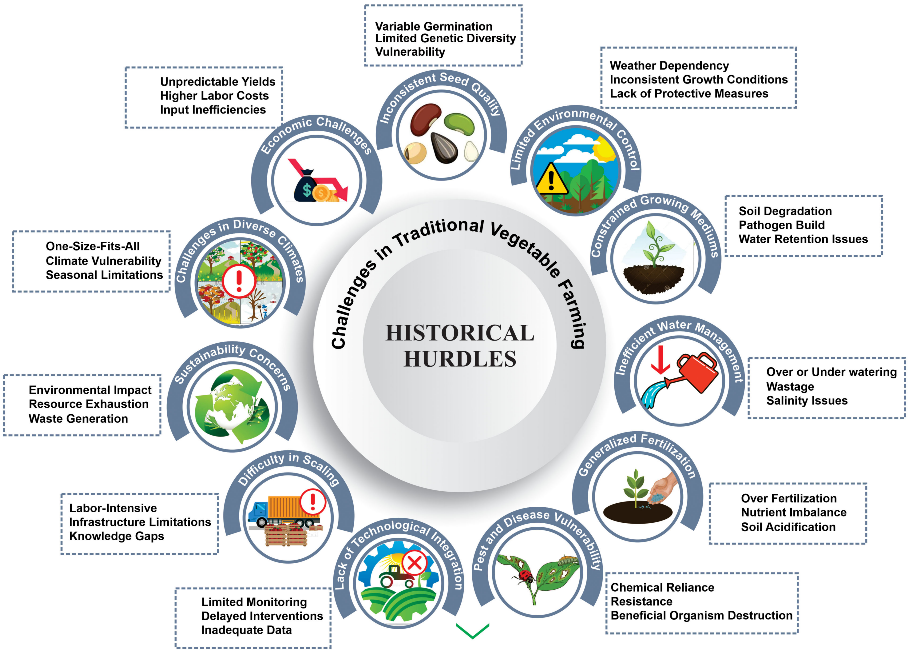

FIGURE 1
Multifaceted challenges in traditional vegetable farming: from seed quality to sustainability concerns.

of agricultural progression, characterized by technological innovations, deepening scientific insights, and a nuanced understanding of plant biology (Figure 2). Since the 1970s, vegetable cultivation has relied heavily on empirical knowledge passed down through generations. During this period, soil nutrition predominantly leaned from the organic compost and manure. Simultaneously, the pest management landscape is largely characterized by botanical solutions, with limited mechanization resulting in a predominant reliance on manual labor (Senesi, 1989; Benelli et al., 2017; Ofuya et al., 2023). The 1980s signaled a notable shift toward modernization especially in developed nations. This era witnessed the growing popularity of plastic trays for seed sowing and the introduction of growth chambers for enhanced germination control. As the agricultural sector expanded, so did the use of synthetic fertilizers and pesticides. An important breakthrough in this decade was the advent of drip irrigation, which promised efficient water management and marked a move toward precision agriculture (Xi et al., 2022; Yang et al., 2023).

By the 1990s, biotechnological innovations had begun to reshape the vegetable cultivation landscape. Seed priming, pelleting, and coating have emerged as novel techniques for ensuring improved seed vigor and consistent germination. The development of tissue culture techniques has been pivotal in enabling the mass production of disease-free plants. Although the foundational work in tissue culture dates back to the mid-1960s, it was not until the 1990s that these techniques were significantly expanded and refined to facilitate large-scale applications (Thorpe, 2007). Concurrently, there has been heightened emphasis on sustainability, with biopesticides and biofertilizers gaining traction as eco-friendly alternatives. Additionally, the role of beneficial microbial interactions in promoting plant growth has been explored (Gulzar et al., 2020; Liu et al., 2021; Raimi et al., 2021).

The turn of the millennium in the 2000s brought about an array of technological marvels. The integration of geographic information systems (GISs) with GPS-enabled farming tools marks a significant transformation in 21st-century agriculture revolutionizing farm management and decision-making processes. This synergy enhances efficiency and sustainability by combining the locational accuracy of GPS with the analytical prowess of GIS. Such integration allows for precise seedling placement and optimized irrigation practices, as evidenced by the deployment of soil sensors and nutrient management strategies (Nyakuri et al., 2022). Moreover, the advent of automation in vegetable farming, highlighted by the introduction of advanced feeding devices and transplanters, aligns with this technological evolution, further contributing to increased efficiency (Dadios et al., 2022). Notably, precision agriculture technologies, like GPS and GIS, have transcended traditional productivity measures offering in-depth insights into soil quality, crop distribution, and environmental conditions. These advancements not only bolster productivity but also reinforce sustainable farming practices, as they enable tailored interventions that cater to the specific needs of different farm sections (Koch and Khosla, 2003; Toscano et al., 2019; Nie and Yang, 2021).

The 2010s strengthened the agricultural sector's commitment to sustainability and innovative farming practices. Vertical farming emerged as a solution to space constraints in urban settings, while techniques, such as hydroponics and aeroponics, revolutionized soilless cultivation (Zhang et al., 2018; Mustapha et al., 2022; Paucek et al., 2023). These advancements have been pivotal in addressing sustainability objectives by investigating water quality and microbial life in hydroponic cultivation contexts (Paucek et al., 2023). Furthermore, the period saw the increasing application of machine learning (ML)-driven diagnostics as essential tools for preemptive pest and disease identification highlighting the role of data-driven learning in agriculture (Tzounis et al., 2017). The burgeoning world of the Internet of Things (IoT) also saw application in real-time farm monitoring and management

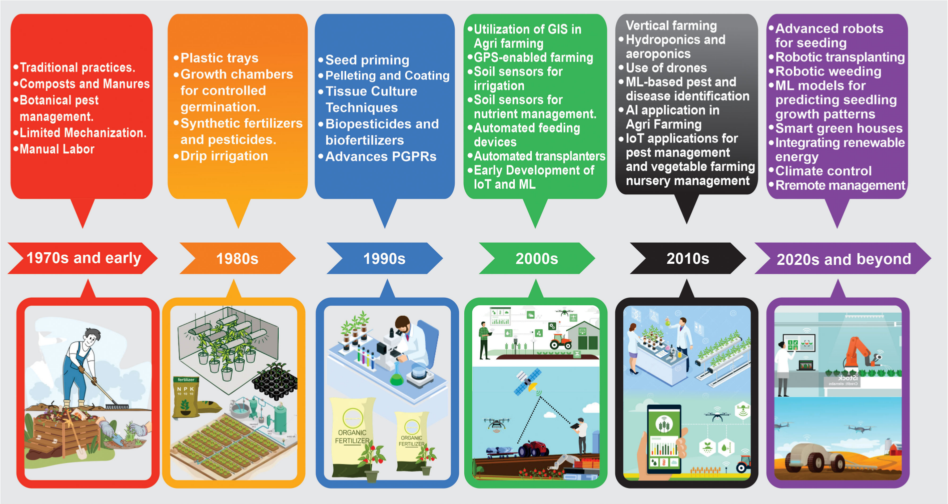

FIGURE 2
Chronological map of transformative techniques and technologies in vegetable cultivation practices from the 1970s through the 2020s. Spanning traditional practices to cutting-edge innovations underscores the rapid evolution of vegetable farming in response to changing agricultural needs and technological advancements.

integrating advanced technologies to enhance agricultural efficiency and productivity (Ahmed et al., 2018; Raj et al., 2021; Namana et al., 2022).

Finally, the 2020s witnessed the emergence of more sophisticated technologies. Robotic systems have begun to perform various farm tasks, including seeding, transplanting, and weeding. ML models provide insights into crop growth patterns and optimized cultivation strategies. Gene-editing techniques, especially clustered regularly interspaced short palindromic repeats (CRISPR), have opened new avenues for improving crop traits. Smart greenhouses, integrating renewable energy sources, advanced climate control systems, and remote management capabilities, have become hallmarks of modern vegetable farming (Deshmukh et al., 2020; Fizikova et al., 2021; Kumar and Prabhansu, 2022; Zhu and Shang, 2022; Furquim et al., 2023).

3 From seed to seedling

3.1 Seed quality, selection, and advanced assessment techniques

The success of vegetable cultivation is fundamentally anchored in the quality and meticulous selection of seeds, a principle that has been an integral part of agricultural practices throughout history (Figure 3). The caliber of seeds sets the stage for the entire cultivation process dictating germination rates, uniformity in sprouting, and synchronized development across the crop. These attributes are critical for ensuring cohesive growth patterns and optimizing the agricultural yield (Pagano et al., 2023). Seed quality is a multifaceted concept that encompasses various attributes, including seedling vigor, which is a composite indicator of seed longevity, germination speed, and early stress tolerance. High-quality seeds are characterized by their ability to produce resilient seedlings capable of withstanding environmental adversities, such as fluctuating temperatures, drought conditions, and prevalent diseases, thereby contributing significantly to the overall robustness of the crop (Croft et al., 2018; Reed et al., 2022).

The genetic integrity of seeds is pivotal for ensuring the desired growth dynamics, resistance mechanisms, and yield potentials in crops. Understanding the metabolic pathways involved in stress responses, particularly the regulation of reactive oxygen species (ROS) homeostasis and DNA damage repair, is crucial for seeds to adapt to environmental challenges (Schieber and Chandel, 2014; Waterworth et al., 2016). ROS serve as crucial signaling molecules in seeds influencing germination and stress adaptation by modulating metabolic and hormone pathways. The interplay between ROS and reactive nitrogen species (RNS) is essential for seed dormancy and germination particularly under stress conditions (Waterworth et al., 2016; Reed et al., 2022). ROS's role extends to plant growth and development, where their balance is key; while they support acclimation to stress and dormancy breaking, excessive ROS levels can lead to cellular damage (Schieber and Chandel, 2014; Waterworth et al., 2016). The implications of seed quality are also economically significant and influence resource allocation and overall productivity. Seeds of superior quality allow for the optimized utilization of water, growing medium, and labor, thereby minimizing waste and maximizing returns. Innovative management programs, such as the Integrated Knowledge and Products Strategy (IKPS), have demonstrated that the application of quality seeds, in conjunction with efficient farming practices, can lead to substantial reductions in

FIGURE 3
Revolutionizing agriculture: modern farming techniques and their multifaceted advancements for sustainable global food production.

resource use and environmental impacts while increasing yield (Wang et al., 2021). Investment in quality seeds provides a robust foundation for successful cultivation promising not only higher yields but also greater environmental and economic sustainability.

The assessment of seed quality has evolved significantly, transitioning from reliance on traditional, empirical methods to the adoption of advanced, scientific techniques. Historically, seed selection was predominantly based on visual inspection, focusing on attributes like size, color, and texture. For example, manual sieve analysis has been employed for grading seeds, such as chickpeas, based on their size (Khatri and Agrawal, 2021). In the modern era, technological advancements have revolutionized seed quality assessment, introducing non-destructive, efficient methods such as optical and multispectral imaging. These cutting-edge techniques allow for a rapid and comprehensive evaluation of seeds' physiological potential and vitality enabling the detection of mechanical damages and other imperfections that are not visible to the naked eye (Salimi and Boelt, 2019; Galletti et al., 2020). Complementing these imaging techniques are biochemical assays, including tetrazolium and TTC tests, which provide insights into the metabolic activity and viability of seeds. These assays can be tailored to specific seed types by adjusting parameters, like exposure times and chemical concentrations, offering a versatile tool for seed quality assessment (Salazar Mercado et al., 2020).

Germination tests remain a cornerstone of seed quality assessment serving as a fundamental and reliable method to gauge seeds' potential to develop into healthy plants. These tests often validate findings from more rapid assays ensuring a comprehensive evaluation of seed quality (Reed et al., 2022). Moreover, innovations, such as X-ray analysis, have opened new vistas in seed assessment allowing researchers and practitioners to peer into the internal structure of seeds. This technique is particularly valuable for identifying internal damages or defects that could impact seed viability and, subsequently, crop yield (MaChado et al., 2020). The genetic integrity of seeds is another critical aspect of seed quality with molecular markers and next-generation sequencing playing pivotal roles in ensuring genetic robustness. These techniques are instrumental in preserving genetic diversity and safeguarding against genetic drift or contamination, thereby maintaining the desired traits in cultivated crops (Salgotra and Chauhan, 2023).

Additional methodologies, like electrical conductivity tests, offer unique insights into seed vigor by quantifying electrolyte leakage providing a proxy for membrane integrity and overall seed health (Ortiz et al., 2018). Pathogen detection has also seen significant advancements with techniques, such as PCR coupled with nanomaterial-based sensors, enabling rapid, accurate identification of seed-borne pathogens ensuring the sowing of healthy, disease-free seeds (Kumar et al., 2021). Hence, the modern approach to seed quality assessment in vegetable cultivation is characterized by a harmonious blend of traditional wisdom and advanced scientific innovations. This comprehensive strategy not only facilitates the selection of superior-quality seeds but also ensures the resilience, health, and sustainability of future crops meeting the complex demands of contemporary agriculture and contributing to global food security.

3.2 Seed treatments and their impact on germination and seedling growth, vigor, and stress tolerance

Seed treatment plays a critical role in enhancing seed quality, optimizing germination rates, and fostering the health of emerging seedlings. These treatments, as illustrated by Ahmed et al. (2019) and Sharma et al. (2015), ensure faster and consistent germination while enhancing resilience against various biotic and abiotic challenges. One primary treatment, seed priming, involves controlled hydration of seeds. Pre-germinative metabolic activities are initiated by soaking seeds in either pure water or specific solutions. This process imbues seeds with vigor leading to resilient seedlings that can better navigate environmental challenges (Raj and Raj, 2019). In addition to priming, seed coating and pelleting have gained prominence. Seeds are enveloped by materials ranging from organic clays to modern synthetic polymers. Some treatments integrate advanced elements, such as nanoparticles, for additional protection. For example, zinc nanoparticles (Zn NPs) used in seed treatments are invaluable given their role in the synthesis of enzymes crucial for germination. Appropriate concentrations of ZnO NPs can enhance germination and vigor (Timilsina and Chen, 2021). As for stress tolerance, seed treatments have been especially vital in countering drought stress (Ahmed et al., 2019). Research on plants, such as *Vicia faba*, indicates that seed priming with specific extracts can trigger genetic adaptations to mitigate the adverse effects of drought (Kasim et al., 2019). Another promising strategy is the application of plant hormones. Hormones, such as gibberellins (GAs), cytokinin, and auxins, can expedite germination, deter pests, and mitigate oxidative stress, thereby leading to healthier plants (Ghafoor et al., 2020; Madany et al., 2020). GA_3 priming enhanced germination and early growth of rapeseed under drought conditions simulated by PEG-6000. These data suggest that the decreased germination and growth in non-primed seeds during drought are due to increased ROS production. Seeds primed with GA_3 showed increased activity of antioxidants, such as superoxide dismutase, peroxidase, catalase, ascorbic acid, and glutathione (Zhang et al., 2022). In certain crops, such as lettuce, seeds primed and then coated with clay-based materials can germinate efficiently even under elevated temperatures, a boon for hotter climates (Valdes and Bradford, 2022). Biopriming offers another innovation avenue. Treating seeds with beneficial microorganisms can enhance germination and overall plant growth. For instance, a blend of certain beneficial microbes proved to be more effective than traditional fungicides in French bean (Negi et al., 2021). Thermotherapy, which subjects the seeds to specific high temperatures, can neutralize seed-borne pathogens, while introducing beneficial microbes to seeds improves nutrient absorption and provides a natural defense against diseases (Ding et al., 2013; Davino et al., 2020). Furthermore, addressing threats, such as the tomato brown rugose fruit virus (*ToBRFV*), involves understanding its presence in seed parts and evaluating different disinfection methods. Chemical pesticides, while effective against pests, such as nematodes, require careful application to preserve seed integrity during germination (Patil et al., 2021). In essence, seed treatments equip seeds for their forthcoming journey, ensuring that they are not only ready but primed for excellence, laying the groundwork for prolific harvests.

4 Role of hardening, acclimatization, and transplantation

In vegetable cultivation, the convergence of traditional practices and advanced methodologies has been prominently demonstrated in the techniques of hardening, transplantation, and acclimatization (Melissas et al., 2022). Young seedlings, although brimming with potential, face inherent vulnerabilities in their formative stages (Bag et al., 2019). The hardening process, which entails gradual exposure of young plants to fluctuating environmental conditions, strengthens them against potential stresses (Bag et al., 2019). Transplantation, on the other hand, aids in their movement to more conducive habitats optimizing root development and plant establishment (Shao et al., 2021). Concurrently, acclimatization acts as a pivotal transition assisting young plants in adapting to sheltered environments, such as greenhouses, and to the vagaries of open terrains (Bag et al., 2019). Collectively, these methods exemplify the synergistic blend of age-old agricultural insights and contemporary vegetable farming practices emphasizing the importance of fostering crop resilience and vitality.

4.1 Seedling hardening and acclimatization

Seedling hardening, a revered tradition in agriculture, is now viewed through the lens of both conventional wisdom and state-of-the-art methods. This phase seeks to bolster seedlings getting them field ready by enhancing their resilience against potential environmental adversities. In the past, hardening was achieved via simple tactics such as modulating water intake and progressively introducing seedlings to sunlight. However, in the modern era, seedlings are immersed in nutrient-rich solutions that offer vital nutrients, such as nitrogen, phosphorus, and potassium, setting them on a path of vigorous growth and efficient energy transfer (Marschner, 2011). However, the growth story does not end with basic nutrients. Growth regulators, such as auxins and cytokinins, are fundamental in refining root development and cellular division. This adaptability enables crops, such as strawberries, to overcome hurdles, such as soil salinity, resulting in an improved photosynthetic rate and better cell structure (Zhang et al., 2021; Padilla et al., 2023). Adapting to the environment's whims is critical. Techniques, such as cold hardening, saline conditioning, and controlled water stress, are pivotal. For instance, cold hardening introduces seedlings to reduce temperatures ensuring resilience against unexpected frost (Kolupaev et al., 2020). The mutualism between seedlings and beneficial microbes has also been highlighted. For instance, mycorrhizal fungi not only deter diseases but also boost nutrient absorption (Aini et al., 2019). Simultaneously, the integration of compost with such microbes has increased drought resistance in crops such as tomatoes (Tahiri et al., 2022). There is growing interest in anti-stress compounds. For example, osmoprotectants counteract the negative impacts of environmental stressors (Ahmed et al., 2021a). In onions, proline has been a game-changer for mitigating drought-induced stress (Semida et al., 2020).

The integration of controlled UV hardening and specific light spectra in seedling development has shown significant promise in enhancing plant growth and resilience. UV hardening, involving exposure to UV radiation doses, stimulates phenolic compound production, a critical factor in plant defense mechanisms (Strid et al., 1994; Teklemariam, 2002). Advancements in light-emitting diode (LED) technology have led to the development of phosphor-converted (PC) white LEDs, which utilize a phosphor layer over blue LED chips to produce white light (Zhao et al., 2019). This process creates a broad spectrum, predominantly in the green–yellow–red range, and is known for its high energy efficiency and cost effectiveness due to mass production (Meyer and Tappe, 2015). However, for precise spectral needs, especially in enhancing plant growth, systems with monochromatic LEDs, offering tailored red and blue light combinations, might be more beneficial (Kong et al., 2019). Monochromatic red and blue LED lights have been proven effective in improving dry shoot biomass, root architecture, and stem diameter of seedlings in both pre- and post-transplantation phases (Kong et al., 2019; Melissas et al., 2022; Zhao et al., 2023). Contrastingly, seedlings exposed to standard fluorescent lamps and isolated red or blue light have shown suboptimal growth especially under blue light (Zhao et al., 2023). The most beneficial light condition, particularly for high-quality grafted tomato seedlings adapting to transplant shock, involves a specific combination of red and blue light (Melissas et al., 2022). In certain artificial lighting configurations, light, primarily in the red wavelength range combined with a specific proportion of blue spectrum radiation, has been found to enhance stem diameter, root development, and the accumulation of phenolics and antioxidants. Furthermore, lettuce grown under alternating red/blue light demonstrated accelerated growth and higher levels of nutrients, like sugars, ascorbic acid, and anthocyanins, suggesting that this lighting regimen can enhance both growth and nutritional quality offering a dual benefit in plant factory settings (Ohtake et al., 2018). The transition to high-tech greenhouse cultivation has been greatly shaped by fluorescent lamps, whose wider spectrum emission offers more flexibility in light manipulation compared to traditional discharge lamps, marking a key advancement in controlled environment agriculture (Brown et al., 1995). Furthermore, the development and continued use of Growlux technology have further revolutionized *in vitro* plant cultivation exemplifying the technological progression toward optimizing light conditions for plant growth especially in contexts where natural light conditions are not sufficient or controllable (Morrow, 2008). The use of fluorescent lamps has been associated with increased MDA contents in shoots indicating their impact on plant growth and development (Astolfi et al., 2012). However, it is important to note that fluorescent lamps emit mercury, which can cause environmental pollution (Li et al., 2018). In contrast, LED lamps have been considered safer to operate and have lower energy consumption compared to fluorescent lamps making them an attractive option for controlled environment cultivation (De

Carvalho et al., 2021). The effects of different light sources, including fluorescent lamps, LEDs, and high-pressure sodium (HPS) lamps, on plant growth and development have been extensively studied. For instance, the use of fluorescent lamps has been found to be effective for displaying flower colors and has been used as the standard condition in some studies (Yang et al., 2014). Additionally, the use of HPS lamps in greenhouse crops has traditionally been the main source of light in winter months, although LEDs are becoming more common in horticulture (Treder et al., 2021).

Additionally, the role of temperature fluctuations in preparing seedlings for varying outdoor conditions cannot be overstated. Such environmental control is crucial in the early stages of plant development for ensuring adaptability and resilience (Chalker-Scott and Scott, 2004; Indergard et al., 2022). Wind exposure also plays a major role in strengthening the seedlings (Gardiner et al., 2016). Biostimulants have earned their place in modern agricultural practice. For example, the application of *Ecklonia maxima* extract has shown dramatic improvements in plant growth, yield, and nutrient quality (Kocira et al., 2018; La Bella et al., 2021; Rakkammal et al., 2023). Physical manipulation, a seemingly simple approach, enhances stem strength proving that the most traditional methods sometimes retain their value (Bag et al., 2019). Blending traditional practices with contemporary techniques equips seedlings with strength and resilience paving the way for thriving agricultural landscapes.

4.2 Automated transplanting: modernizing vegetable farming practice

Transplanting stands as an age-old rite in agriculture, a practice that is steeped in tradition and skill. In its earliest form, transplanting was a manual, labor-intensive task that required an artisanal touch to mitigate root damage and prevent plant shock. With time and technological advancements, semi-automatic transplanters have emerged offering a perception of mechanization (Kacheyo et al., 2023). However, their efficiency often waned with operator fatigue leading to occasional inconsistencies in plant spacing and depth. This landscape has experienced a seismic shift with the dawn of automation in agriculture. Today's automated transplanters, which merge cutting-edge technology with agrarian principles, promise both speed and precision (Khadatkar et al., 2018). These systems prioritize the delicate handling of plants, ensuring that root structures remain intact, and setting the stage for swift acclimatization in their new homes. By incorporating sensor technology, modern transplanters offer real-time feedback, which equips farmers with the ability to make instantaneous adjustments for optimal transplantation outcomes (Jin et al., 2019). Robotic systems with vision sensors or end effectors represent the pinnacle of this evolution. Individual seedlings were meticulously selected from trays to ensure precise placement at predetermined sites. Such systems typically combine an array of specialized components, such as vision systems, grippers, manipulators, and drum-type seedling removal devices, reflecting the harmony of precision and efficiency (Khadatkar et al., 2018). Research has underscored the multifaceted benefits of automated transplantation. Jin et al. (2019) emphasized the role of automation in minimizing transplant shock, which is a critical factor in determining seedling survival and subsequent growth. Furthermore, as Khadatkar et al. (2018) pointed out, automated systems have the potential to reduce operational costs in the long run while also consistently ensuring that seedlings are planted at optimal depths and spacings. Another compelling benefit, as highlighted by Christiaensen et al. (2021), is the potential reduction in labor shortages during peak planting seasons, which can profoundly impact crop yield and quality. The fusion of time-honored practices with technological innovations remains evident in this transformative journey from manual transplantation to advanced automation. Such advancements not only honor the essence of agriculture but also pave the way for a more efficient and sustainable future.

5 Soil and growing media innovations

5.1 Introduction to traditional soil and growing media

The success of vegetable cultivation is deeply anchored in the meticulous selection of the soil and growing media (Figure 3). Natural soil has historically been the cornerstone of vegetable cultivation sustaining agriculture for millennia by providing an organically rich habitat for plants. However, this reliance is not without challenges, including susceptibility to diseases, pest infestations, and potential nutrient deficiencies (Olle et al., 2012; Abd-Elgawad, 2019). Historical solutions to these challenges often involve the use of compost and other similar media. Compost, derived from decomposed organic matter, revitalizes soil by supplying a mix of nutrients and beneficial microorganisms while also enhancing soil structure (Zinati, 2005). As the 20th century dawned, the introduction of biocontrol agent-fortified composts amplified the disease-suppression capabilities inherent in traditional composts (Zinati, 2005). Peat moss, often paired with biochar, has become another popular growing medium lauded for its exceptional moisture retention and pH-balancing properties, which together create an optimum environment for root development (Bachmann et al., 2018; Vaughn et al., 2021). The course of growing media underwent a transformative shift with the advent of innovations, such as soilless cultivation methods, including hydroponics and aeroponics, which promised superior yields and expedited growth rates. Coir, a byproduct of coconut processing, has emerged as an eco-friendly alternative to peat moss (Mariotti et al., 2020). Concurrently, modern agriculture embraces bio-fertilizers, which introduce beneficial microorganisms to aid plants in nutrient uptake, thereby reducing their dependency on synthetic fertilizers (Fertahi et al., 2021; Raimi et al., 2021). Typical substrate components include minerals in natural or modified forms, such as sand, lava rock, expanded shale, clay, and slate; recycled waste materials, such as crushed bricks or tiles; crushed or aerated concrete and subsoil; and stabilized organic matter, such as composts, plastic materials, and slow-release fertilizers. Incorporating technology, sensors have begun to offer real-time data on the moisture, nutrients, and pH levels of the media, paving the way for precision agriculture. Leading-edge research has ventured into the potential of 3D printing and nanotechnology aiming to optimize and enhance the properties of growing media. Responding to the increasing demand for organic produce, organic-growing media devoid of synthetic additives made their mark. Furthermore, the emphasis leaned toward developing disease-suppressive media by integrating beneficial microbes consequently decreasing the dependence on chemical fungicides. Addressing specific plant requirements, the industry has witnessed a trend toward custom-designed growing media tailored exclusively for individual crops (Ampim et al., 2010; Przemieniecki et al., 2021; Antonious et al., 2023; De Marco et al., 2023). Drawing on the rich tapestry of time-tested approaches to soil and growing media, the current wave of breakthroughs and innovations signals a promising horizon. The continuous evolution of growing media, intertwining age-old wisdom with the forefront of scientific discovery, is poised to revolutionize agriculture addressing both the burgeoning needs of the global population and the pressing call for sustainable farming practices.

5.2 Advancements in soilless culture

Hydroponics, a novel technique for growing plants without soil, has significantly altered the landscape of controlled environment agriculture. Reports suggest that various aspects, such as dry matter, sugar, soluble solids, vitamins, and carotenoid content in crops, such as tomatoes, are superior when cultivated through soilless systems than in traditional soil (Olle et al., 2012). By replacing soil with nutrient-rich water, hydroponics offers numerous benefits, including increased nutrient absorption, faster growth rates, and elimination of many soil-borne diseases (Waiba et al., 2020). The journey began with basic hydroponics in which plants were grown in static nutrient-infused water (Maucieri et al., 2019). The convergence of hydroponics with recirculating aquaculture systems is a sustainable method for aquaponics. The choice of hydroponic technology in aquaponics depends on various factors, including environmental conditions, financial viability, crop type, and spatial availability (Maucieri et al., 2019). As this field progressed, innovative systems, such as the nutrient film technique, emerged, marked by a continuous flow of nutrient solution, making it especially useful for crops with shorter growth cycles, such as lettuce (Alipio et al., 2019). Another remarkable addition, deep-water culture, sees plants submerged in aerated nutrient solutions making it suitable for leafy greens (Nursyahid et al., 2021). Vegetable cultivation, from its primitive origins in foraging to the advanced vegetable science of today, has continually evolved. This discipline has not only developed high-yielding and nutritious vegetable hybrids adaptable to varying conditions but also devised techniques to counter climate adversities. These innovations extend to controlled environmental farming and strategies against climate change (Singh, 2023). Another advancement, aeroponics, maintains plant roots in the air intermittently misting them for nutrients. This approach maximizes access to oxygen, promotes growth, and reduces disease risk (Singh, 2023). Simultaneously, the drip system, utilizing intricate tubing and drip emitters, allows for the precise delivery of nutrients ideal for larger or specific plant species (Yang et al., 2023). Alternate growing media, such as coco coir and a blend of peat and perlite, although still considered hydroponic, have gained traction because of their impressive moisture retention and aeration capacities (Mariotti et al., 2020). Aquaponics, merging aquaculture, and hydroponics represent an epitome of sustainability, with fish waste providing nutrients for plants, which in turn purifies water for fish (Maucieri et al., 2019). Modern hydroponics is further augmented by technological advancements enabling automated monitoring and adjustment of parameters essential for plant growth, such as nutrient balance, pH, and temperature (Joshitha et al., 2021). Hydroponics, transitioning from its basic beginnings to a discipline enriched by technology, showcases the promise and potential of soilless cultivation in the future of global agriculture.

5.3 Importance of pH, electrical conductivity, and nutrient balance in growing media

The growth and prosperity of plants within a growing medium hinge on a myriad of interconnected conditions. The list of these essential factors is the pH level, electrical conductivity (EC), and overall nutrient balance present within the medium. Ensuring the optimization of these parameters is crucial for achieving peak plant health and yield. These findings have been consistently underscored across a range of studies affirming the foundational significance of these parameters in agriculture and horticultural farming systems (Marschner, 2011; Xiong et al., 2017; Fan et al., 2020). In greenhouse cultivation, maintaining the appropriate pH level of the substrate is crucial as it directly influences nutrient uptake by plants. A neutral pH level is marked at 7 on the scale with values below indicating acidity and above denoting alkalinity. Optimal nutrient absorption often requires precise pH adjustments to align with the specific needs of the plants being cultivated (Marschner, 2011; Xiong et al., 2017). Within intensive systems, such as plastic greenhouse vegetable production (PGVP), there is a risk of excess nutrients. Obsessive fertilization can trigger soil degradation compromising long-term usability. An illustrative study highlighted the nutrient surplus within PGVP, hinting at potential pitfalls, such as rapid soil nutrient build-up, acidification, and secondary salinization (Fan et al., 2020). EC is a pivotal benchmark for growing media. It represents the volume of dissolved salts, which is a proxy for nutrient concentration. EC media provide vital clues on whether plants are receiving optimal nourishment. Varying substrates, each with unique characteristics, can influence the EC, pH, and nutrient dynamics differently. Notably, as eco-consciousness rises, the quest for sustainable substrates has gained momentum. In this regard, alternatives, such as coconut coir, have shown promise rivaling traditional choices such as rockwool and peat vermiculite (Xiong et al., 2017). An ideal pH is slightly acidic for a diverse vegetable palette typically falling between 6.0 and 7.5. In vegetable-producing regions, critical water parameters, such as nitrate, phosphate, and total dissolved solid concentrations, require keen attention. The Best Management Practices strive to harmonize nutrient inputs and protect water sources while ensuring sustainable yields (Liu et al., 2022a). The pH spectrum intertwines with the soil microbial community impacting nutrient dynamics and bolstering plant health (Husson, 2013; Bárcenas-Moreno et al., 2022). The oxidation–reduction potential (Eh) is a dimension that is yet to be extensively studied. In addition to pH, Eh can reshape soil, plant, and microbial interactions. Groundbreaking research posits that an interplay between Eh, pH, and biological activity could revolutionize cropping strategies (Husson, 2013). Plant health is a nuanced ballet of multiple factors arranged within their growth medium with pH, EC, and nutrient balance as lead regulators. pH regulates nutrient gates, EC reflects nutrient content, and nutrient balance ensures collaborative functionality (Marschner, 2011; Husson, 2013). However, a misstep can disrupt homeostasis. As emphasized by researchers, these parameters are not mere numerical indicators, but play a crucial role in determining the overall performance and yield of crops (Bárcenas-Moreno et al., 2022; Liu et al., 2022b). Comprehending these elements gives one the ability to seamlessly orchestrate the entire growth process transitioning a plant from its nascent seedling stage to mature fruition. It is imperative to understand the nuances of pH, EC, and nutrient balance for those striving for maximum growth and yield. Based on this knowledge, growers can ensure optimal plant development and guide them to achieve their maximum potential.

6 Nutrition and water management in agriculture farming system: a vital shift toward sustainability

Thus, the importance of agriculture cannot be overstated. It not only feeds our burgeoning global population but also serves as a fulcrum for the ecological balance of our planet teeters. One of the linchpins of this balance is the nutrition management in agriculture (Figure 3). Traditionally, farmers have leaned heavily on broad-spectrum fertilizer applications. Although these methods deliver yields, they often result in significant over-fertilization, which, in turn, has a cascade of environmental repercussions. These include challenges, such as soil acidification, increased soil salinity, and the dire concern of heavy metal contamination, as detailed by Mikula et al. (2020). However, as pointed out by Tripathi et al. (2022), plants, much like humans, require balanced nutrition. This balance is a delicate dance of nutrients that, if skewed, can greatly affect plant health, growth, and resilience to diseases. Nutrients, such as nitrogen and potassium, have proven instrumental in bolstering plant defenses. Although nitrogen facilitates the amplification of defense-related enzyme levels, potassium plays a pivotal role in enhancing plant polyphenolic concentrations both of which are essential for defense mechanisms. Conversely, elevated phosphorus levels might increase the vulnerability of plants emphasizing the intricacy and delicacy of nutrient management.

Enter precision agriculture: a paradigm shift that could not have come at a more crucial time. Harnessing technological advancements, this approach, as elucidated by Bar-Yosef (2020), reported the precise provision of nutrients. This ensures that plants receive the exact quantities of nutrients they need. Such an approach not only optimizes yield but also dramatically reduces waste and mitigates the adverse environmental impacts associated with traditional farming methods. One of the most pressing areas of concern underlined by both Bar-Yosef (2020) and Mikula et al. (2020) is micronutrient management. Although indispensable, these nutrients can be toxic in excessive quantities. Metallic microelements, such as copper, iron, and zinc, if mismanaged, can concentrate in the root zone, which has detrimental effects on plants. Additionally, Tahat et al. (2020) elucidated the pivotal role of soil health assessment in nutrition management. A comprehensive understanding of soil quality, including the bioavailable forms of macro and micronutrients, paired with an assessment of weather conditions, can guide farmers in making precise decisions regarding fertilization (Rodríguez et al., 2022). This integrative approach ensures that the soil, the bedrock of agriculture, remains fertile and free of contamination. Facing the dual challenges of feeding a growing population and environmental conservation, advanced nutritional management in agriculture is crucial. Sustainable farming is now essential, and precision agriculture, which focuses on accurate nutrient allocation, offers a promising way forward showcasing the power of innovation for a sustainable future.

6.1 Organic nutrient usage in modern agriculture

In the contemporary age of agriculture, where sustainability is a clarion call, organic nutrients stand as a beacon for harmonizing farming practices with ecological necessities. Rooted in nature and eschewing the toxicity associated with many synthetic compounds, organic fertilizers embody the nexus between tradition and innovation. In natural cycles and ecological systems, organic farming hinges on the cyclical reintroduction of organic matter into the soil rejuvenating its fertility. This organic mantra transcends mere philosophical postulations and finds resonance in empirical evidence. Research has shown that organic fertilizers sourced from decomposed organic waste, plant residues, or animal byproducts significantly elevate the organic content of the soil and optimize it for sustained agricultural productivity (Shaji et al., 2021). Aside from being ecologically friendly, these products have demonstrated their advantages in enhancing soil texture, water retention, and microbial life (Fahrurrozi et al., 2019; Liu et al., 2022a; Liu et al., 2022b). Such soil improvements are pivotal forming a bedrock upon which healthier and more resilient crops thrive. Diverse in their constitution, organic fertilizers encompass a wide spectrum, from compost, a ubiquitous byproduct of decay, to more specialized derivatives, such as blood meal, fish emulsion, and seaweed extracts. Notably, advancements in the organic realm, such as the advent of controlled-release fertilizers (CRFs), have enabled sustained nutrient delivery merging the advantages of both organic and inorganic fertilizers. These fusions promise both nutrient enrichment and the reduction of environmental hazards (Shaji et al., 2021). However, the merits of organic nutrients have not been concealed. As reported by Ahmed et al. (2021b), crops cultivated using organic fertilizers, such as strawberries, often surpass their conventionally grown counterparts in terms of growth, nutrient content, taste, and longevity in shelf life. This quality enhancement is not an isolated incident; numerous studies have corroborated the superior nutrient profiles and health benefits associated with organically grown produce. However, the most resonant attribute of organic nutrients lies in their contribution to sustainable agricultural models. By championing biodiversity, these nutrients foster a vibrant soil microbiome that is integral for disease resistance and optimal nutrient uptake (Nikolaou et al., 2023). Furthermore, the incorporation of organic waste products, such as compost and manure, epitomizes the principles of a circular economy, minimizes waste, and ensures that natural cycles are respected and replicated.

6.2 The role of slow-release fertilizers in plant growth

A strong start in the early seedling phase not only ensures robust growth but also bolsters resistance against potential challenges. Slow-release fertilizers (SRFs) have emerged as pivotal players in ensuring that this critical phase of plant growth is optimally supported. One of the most innovative contributions to the annals of farming is that SRFs provide a nuanced understanding of plant nutrition. Unlike conventional fertilizers that offer a rapid, often excessive, dose of nutrients, SRFs gradually release these vital components. This modulated release, achieved through sophisticated encapsulation mechanisms or microbial degradation, ensures that plants receive a steady flow of nutrients (Nardi et al., 2018; Shaji et al., 2021). Such a sustained nutrient supply is instrumental in fostering the development of a robust root system, which is pivotal for the overall health and resilience of plants in later stages (Wang et al., 2020). The inception of nanofertilizers, particularly formulations, such as coated nanourea, accentuates the fusion of technology with traditional farming practices (Sharma et al., 2022). The observed enhancement in parameters, such as root and shoot length, photosynthetic pigments, and antioxidative capabilities in plants treated with nanourea/chitosan nanocomposites, as opposed to bare nanourea or commercial urea, indicates the potential of these novel fertilizers. However, their efficacy is intricately tied to the dosages applied, underscoring the need for precision in deployment. SRFs, while replete with their advantages, are not solutions. Their efficient utilization demands a comprehensive understanding of their interactions with environmental variables, compatibility with irrigation systems, and potential long-term impacts on soil health. However, the rewards, particularly for the early seedling stage, were significant. Not only do they sidestep the pitfalls of over-fertilization but their controlled release also ensures optimal nutrient assimilation laying the foundation for healthier, more vigorous crops (Fertahi et al., 2021). In the grand tapestry of sustainable agriculture, SRFs represent a thread of innovation, weaving scientific advancements together with age-old agricultural wisdom. As the push for sustainable, efficient, and eco-friendly farming practices has gained momentum, the role of slow-release fertilizers in nurturing the future, one plant at a time, is poised to be pivotal.

7 Pest management

7.1 The imperative of integrated pest and disease management

Agriculture is at a crossroads; on one hand, there is pressure to increase production to feed a growing global population; on the other hand, there is an urgent need to adopt sustainable practices to protect our planet and ensure that future generations can meet their needs. This delicate balancing act is evident in how pests and diseases that threaten crops are dealt with. Historically, the agricultural sector has heavily leaned from chemical pesticides. Their efficacy in decreasing pests and ensuring consistent yields has made them popular choices. However, over the years, environmental degradation and health risks associated with their overuse have become apparent (Lalruatsangi, 2021). Nurseries, which are vital to the agricultural supply chain, are particularly sensitive to these challenges. As breeding grounds for future crops, they require vigilant protection from pests and diseases; however, the methods used must not compromise plant health or the surrounding environment (Yadav et al., 2018). This is where IPM is performed. It is not just a method; it is a philosophy that integrates diverse agricultural practices ensuring that they work in tandem to control pests while minimizing environmental impact (Nayak et al., 2021). The true genius of IPM lies in its adaptability and reliance on a deep understanding of its ecological relationships. One of the core tenets of IPM is the use of biological controls essentially leveraging nature's own mechanisms to control pests. From ladybugs to beneficial nematodes, these natural warriors help maintain balance ensuring that pests do not infest crops (Sasanelli et al., 2021). Yellow light has been widely studied for its effectiveness in controlling insect pests. Research has shown that yellow light traps are effective in capturing a variety of insect pests, including rice plant pests, tea green leafhoppers, and tomato leaf miners (Shimoda and Honda, 2013; Pezhman and Saeidi, 2018; Shi et al., 2021). The effectiveness of yellow light traps in capturing and monitoring insect pests has been a subject of interest in IPM strategies offering a non-invasive and environmentally friendly approach to pest control (Reddy, 2011). These studies collectively demonstrate the potential of yellow light traps as a valuable tool in the management of insect pests in agricultural settings.

Moreover, IPM stresses the importance of non-chemical interventions, such as crop rotation, intercropping, and the use of pest-resistant varieties (Figure 3). These methods not only deter pests but also enrich the soil and promote biodiversity (Paudel et al., 2020). However, for chemical interventions, IPM adopts a pragmatic approach. Chemicals are not entirely off the table, but are used sparingly, selectively, and only when other methods prove insufficient. This approach minimizes the risk of pesticide residues in food and the environment (Paudel et al., 2020). An exciting aspect of IPM is the potential of botanical pesticides. Plants, such as neem, garlic, and tobacco, have traditionally been used in various cultures to prevent pests. With modern research validating many of these traditional practices, there is renewed interest in harnessing their potential in a systematic manner. Not only are these plant-based interventions effective, but they also reduce the environmental footprint of agriculture (Lalruatsangi, 2021). As the world becomes increasingly aware of the ties between farming, well-being, and our planet, IPM stands as a guiding light. By merging scientific insights into age-old practices and environmental understanding, IPM charts the way toward a sustainable agricultural horizon.

7.2 Biological control agents and their role in IPM

The significance of IPM has been steadily gaining traction in the evolving landscape of agricultural and horticultural practices. Central to this is the concept of biological control, a practice that offers a more sustainable and environment-friendly alternative to the extensive use of chemicals (Jeffers and Chong, 2021). Entomopathogenic fungi (EPF) have emerged as one of the forerunners of non-chemical insect control. Their modus operandi revolves around acting as biological control agents presenting a potent combination of cost effectiveness, promotion of biodiversity, and a minimal environmental footprint. Furthermore, EPFs have the innate ability to inhabit plants as endophytes. This dual role, where they act both as agents of pest and disease control, and as promoters of plant growth, places them at the forefront of cutting-edge IPM strategies (Mantzoukas and Eliopoulos, 2020). The cultivation of flowering dogwood (*Cornus florida* L.) presents an illustrative example of the challenges faced and potential solutions offered by biological control. Powdery mildew disease is a significant concern. However, the discovery of the endophytic bacterium IMC8 offers a glimmer of hope. Demonstrating resilience across a range of conditions and compatibility with conventional fungicides, IMC8 stands out as a promising contender for powdery mildew. Through its production of volatile compounds with antifungal properties and evident parasitic activity against mildew, this bacterium exemplifies innovative and effective biological control solutions (Rotich et al., 2020).

Exploration of indigenous microbial communities in aquaponics as potential biocontrol agents offers a promising avenue for future research (Folorunso et al., 2021). In the diverse world of biological control agents, one encounters a range of organisms, each offering unique methods to combat pests. Predators, such as lady beetles and lacewings, actively hunt and reduce pest populations. In contrast, parasitoids, such as braconid wasps, employ more nuanced techniques. There are pathogens and competitors, exemplified by organisms, such as *Bacillus thuringiensis* (Bt), which challenge pests by introducing diseases or outcompeting them for resources. The overarching benefits of biocontrol span from environmental to economic. However, it is essential to acknowledge challenges, including ensuring successful establishment, potential side effects on non-target organisms, and navigating variable environmental conditions (Desurmont et al., 2018; Hewlett et al., 2019; Rotich et al., 2020; Nechols, 2021). The adoption and adaptation of biological control agents in IPM strategies represent a paradigm shift in modern agricultural practices. While the promise of a sustainable and environmentally conscious future is palpable, it is imperative to approach this transition with comprehensive research, informed understanding, and thoughtful implementation.

8 Plant growth regulators

Plant growth regulators (PGRs), whether naturally occurring or synthetic, play a transformative role in botany and horticulture profoundly impacting various stages of plant growth and development (Soni et al., 2022; Asghar et al., 2023). These potent compounds, categorized into older groups, such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene, and newer groups encompassing hormones, such as jasmonates, salicylic acid, brassinosteroids, and polyamines, have been observed to have widespread applications in amplifying crop production. When applied judiciously, even in small amounts, PGRs can induce rapid phenotypic alterations in plants influencing their growth trajectory from germination to senescence (Asghar et al., 2023).

8.1 The role of plant growth regulators in vegetable farming

In the intricate choreography of vegetable farming, PGRs have emerged as pivotal players of both natural and synthetic origins (Soni et al., 2022). Their ability to invoke rapid phenotypic changes, even in minute quantities, and their profound influence on plant growth stages from germination to senescence, have garnered significant attention among vegetable farmers (Figure 3). Vegetable farming, a sector perpetually searching for enhanced productivity and quality, has especially benefited from PGRs. As highlighted by Soni et al. (2022), their applications span a wide range from seed soaking to inflorescence spraying. Furthermore, they are instrumental in hybrid seed production, improving seed germination vigor, and enhancing resistance against pests, diseases, and adverse growth conditions ultimately resulting in both qualitative and quantitative yield enhancements. A cornerstone of this growth narrative is the gibberellins, particularly noted for their role in breaking seed dormancy. This translates to accelerated seed germination, which is vital for vegetable seedlings and their subsequent timely transplantation into fields. Additionally, GA_3-primed seedlings showed elevated resilience with improved antioxidant defense mechanisms facilitating better survival under stressors, such as drought (Zhang et al., 2022). Auxins, another subset of PGRs, are indispensable for vegetable cultivation because of their ability to foster robust root initiation, a fundamental requirement for healthy seedlings destined for transplanting. They not only stimulate root growth but also, as recent molecular insights suggest, influence embryonic fate in plants, enriching crop outcomes (Asghar et al., 2023). Although the primary focus of PGRs in vegetable farming has been growth promotion, it is pivotal to recognize their multifaceted roles. For instance, abscisic acid

(ABA) empowers crop plants with enhanced drought resistance and regulates stomatal dynamics, a crucial adaptation for nurseries in arid regions or during water-scarce periods (Chen et al., 2020). However, vegetable farming demands a thorough understanding of the nuanced effects of PGRs. Despite its natural presence in plants, ethylene can expedite leaf senescence, which may not always be desirable in a vegetable farming system (Peerzada and Iqbal, 2021). Hence, precision in the application and awareness of environmental contexts is vital. Over-application can lead to toxicity, and staying abreast with regulatory guidelines is essential. For optimal vegetable farming and enhanced growth leading to better yields, a thorough grasp and wise use of PGRs, as highlighted by research such as that by Soni et al. (2022) and similar studies, is crucial.

8.2 Innovations in the application of PGRs for enhanced crop growth

In recent years, the methods used to apply plant growth regulators (PGRs) in agriculture have undergone revolutionary changes spurred by advancements in technology and deeper botanical insights. Traditional methods, such as soil drenching and foliar sprays, which once dominated the industry, have given way to more sophisticated and effective techniques (Bista et al., 2022; Kuts et al., 2023). A breakthrough in this field was demonstrated in the study of onions. When specific concentrations of the growth regulators GA_3 and NAA were applied at precise stages of the onion life cycle, there was a marked enhancement in attributes such as plant height, number of leaves, and bulb diameter. Specifically, a regimen of 150 mg L^{-1} of NAA at the three-leaf stage and 150 mg L^{-1} of GA_3 at the seven-leaf stage yielded the most promising results (Bista et al., 2022). In line with these discoveries, Kuts et al. (2023) discussed the potential of both synthetic and organic PGRs in modern vegetable cultivation. In particular, they underscored their role in increasing productivity, enhancing product quality, and bolstering resilience against environmental challenges. For instance, when cucumbers grown in film greenhouses are treated with specific growth regulators and microfertilizers at various stages, there is a significant increase in fruit yield.

Seed priming is central to these innovative application methodologies. PGR-rich solutions were used to soak the seeds prior to sowing. This resulted in increased germination rates and enhanced root vigor. This priming has shown remarkable effects on pepper seeds. A factorial experiment indicated that priming seeds with specific concentrations of GA_3 and NAA significantly improves the germination and growth characteristics of various pepper cultivars (Tombegavani et al., 2020). A further innovation frontier is the creation of PGR-embedded slow-release pellets. These pellets utilize materials, such as carboxymethyl cellulose, chitosan, and polylactic acid, to encapsulate PGRs ensuring a slow and steady release of these crucial growth enhancers to plants (Badgar et al., 2022). Complementing this is the rise in nanoencapsulation. By encapsulating PGRs in nanoscale carriers, these growth agents are protected from premature degradation, thereby providing crop plants with a longer supply of nutrients (Zaim et al., 2023). Technological advances, such as electrostatic spraying, have also been incorporated to ensure the even and precise application of PGRs (Zaim et al., 2023). In tandem with these developments, biostimulants, although distinct from traditional PGRs, show promise for augmenting plant growth. Another significant leap is the aeroponic method, wherein PGRs are incorporated into nutrient mists showing the extent to which technological innovation has been interwoven into horticulture. However, while these advancements offer numerous benefits, they also face challenges particularly in terms of the costs and expertise required for their application. Despite these hurdles, the overarching consensus suggested by Sajjad et al. (2017) is that the future of PGR applications in agriculture is bright, with these innovative techniques poised to significantly elevate crop health and productivity.

9 Technological innovations in vegetable cultivation

The dawn of the 21st century has witnessed a transformative phase in vegetable cultivation spurred by groundbreaking technological innovations. Advanced imaging techniques for meticulous crop monitoring (Ampatzidis and Partel, 2019) with the introduction of drones and robotics ensure precision in operations (Warner et al., 2022). The integration of modern technologies reshapes the fabric of horticultural practices (Figure 4). Furthermore, as greenhouses harness the prowess of automation for optimal environmental control (Shamshiri et al., 2018b), the promise of a sustainable and efficient agricultural future becomes apparent.

9.1 Harnessing rainwater and solar energy for sustainable growth

In the dynamic domain of horticulture, contemporary greenhouses have transformed from mere sunlight enclosures to epitomes of avant-garde technology and stringent environmental regulations (Koukounaras, 2021). These facilities have transcended their passive origins and evolved into responsive hubs designed to meet diverse plant requirements. Embodying the attitude of sustainability, modern greenhouses have championed initiatives, such as rainwater harvesting and cyclical water use, fortifying their ecological credentials (Oweis, 2022). The linchpin of this metamorphosis is the fusion of progressive technologies with judicious environmental management. A paramount example is a dye-sensitized solar cell (DSSC). These cells adeptly reconcile energy consumption with optimal sunlight dispersion making their niche an economical and versatile solution in an era sensitive to energy utilization (Koukounaras, 2021). Unlike some traditional solar technologies that inadvertently impede plant growth within greenhouses, DSSCs ingeniously modulate the solar input to favor plant development (Figure 4).

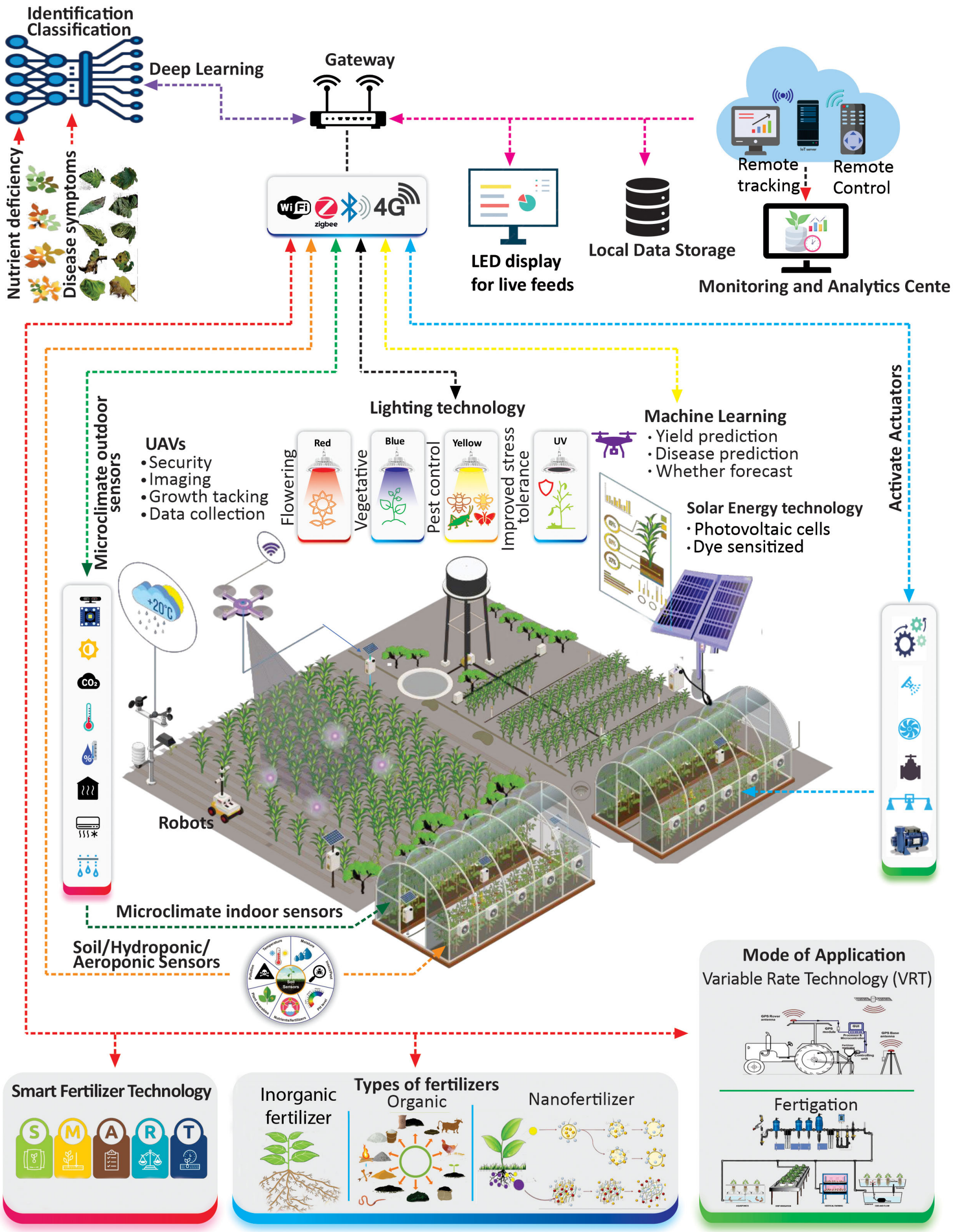

FIGURE 4
This illustration encapsulates the integration of cutting-edge technologies into modern greenhouse vegetable farming. From aerial monitoring by drones to the depth of soil microbiomes, each advancement, represented by nodes, collaboratively optimizes plant growth, health, and productivity. Seamless synergy between natural processes and innovative technologies illuminates the future of sustainable and precise agriculture.

9.2 LED technology and automation: revolutionizing horticultural productivity and sustainability

In recent decades, LED technology has been at the forefront of lighting innovation in plant cultivation. Automated lighting systems are equipped with daylight sensors that gauge natural light levels and supplements, as needed (Barceló-Muñoz et al., 2022). Advanced LED systems allow spectrum control tailored to specific growth stages such as blue light for vegetative growth or red light for flowering (Figure 4) (Kim et al., 2020). Moreover, automatic adjustments protect plants from light stress ensuring that they receive optimal illumination (Paradiso and Proietti, 2022). Kim et al. (2020) highlighted the potential of intra-canopy LEDs in improving the quality and yield of off-season tomatoes. Compared with traditional high-pressure sodium (HPS) lamps, tomatoes grown under specific LED wavelengths exhibited improved physicochemical attributes and increased mineral content. Moreover, LEDs have proven to be more energy efficient underscoring their value in modern agriculture. On the sustainable innovation front, technologies, such as dye-sensitized solar cells and advanced LED lighting, as advocated by

Koukounaras (2021), suggest a harmonious blend of productivity enhancement while curbing agriculture's environmental footprint. Drawing parallels, the research presented by Shadrin et al. (2019) on the niche of vegetable farming exhibits similar trends.

9.3 The role of automation in modern greenhouse cultivation: climate control and CO_2 enrichment

Advancements in greenhouse technologies have dramatically altered the plant cultivation landscape particularly through the integration of automation in climate control. Automated systems, including ventilation, air conditioning, and lighting, are tailored to meet the specific requirements of different plant species (Shamshiri et al., 2018b; Hemming et al., 2019). For instance, automated ventilation systems with roof and side vents respond dynamically to changes in temperature and humidity ensuring optimal airflow necessary for plant health (Fu et al., 2023). Variable-speed fans also contribute to adaptable cooling and air circulation aligning with the current microclimate needs (Hemming et al., 2020). In regions with extreme weather conditions, air conditioning systems play a crucial role in providing precise control over temperature and humidity, which is essential for maintaining the desired levels of CO_2 and other gases within the greenhouse (Colantoni et al., 2018). Solar greenhouses exemplify the synergy between natural ventilation and automated climate control, where the balance between internal and external environments is vital for energy efficiency (Chen et al., 2018; Fu et al., 2023). The interaction between outdoor wind speeds and automated vent openings is essential for achieving desired ventilation rates and regulating indoor temperatures (Chen et al., 2018; Jiang et al., 2023). Additionally, sophisticated automated systems, such as heat pumps and radiant floor heating, modulate their output based on real-time climate data to sustain ideal growth temperatures while enhancing energy conservation efforts (Han et al., 2022; Fu et al., 2023). These precise climate control mechanisms are indispensable not only for warmth but also for managing the heat generated by in-greenhouse equipment, which can collectively contribute to overheating if not adequately regulated. Furthermore, the strategic implementation of CO_2 enrichment has become a cornerstone of modern greenhouse automation. Advanced monitoring systems ensure that CO_2 concentrations are kept at optimal levels enabling plants to maximize their photosynthetic potential while conserving CO_2 resources (Gao et al., 2010; Achour et al., 2020; Huber et al., 2021). Collectively, these technological innovations signify a shift from manual interventions to automated solutions that not only reinforce sustainable farming practices but also enable precise interventions for various plant requirements.

9.4 The emergence of precision fertilization techniques

Precision fertilization, blending technology, and scientific expertise offer innovative solutions to the challenge of harmonizing sustainable agricultural practices with enhanced yields (Figure 3). The core of this approach lies in soil testing ensuring that soil health parameters are understood and interpreted to drive effective fertilization (Mikula et al., 2020; Podar and Maathuis, 2022). Building on this foundation, variable-rate technology (VRT) integrates GPS systems charting the way for tailored fertilization strategies that can vary within a single field (Kumar and Nirosha, 2023). Such granularity in nutrient management is further emphasized through fertigation and foliar fertilization, both of which present avenues for direct nutrient delivery resulting in efficient fertilizer use and rapid responses to nutrient deficiencies (Ahmad et al., 2018; Bar-Yosef, 2020). Furthermore, controlled-release fertilizers (CRF) gain traction, and their ability to maintain calibrated nutrient release emerges as a boon ensuring that plants receive a balanced nutrient supply while simultaneously reducing potential environmental impacts (Cole et al., 2016). Remote sensing and drone technologies are poised to reshape precision agriculture. The exceptional spatial resolution and data-capture capabilities of unmanned aerial vehicles (UAVs) offer real-time insights enabling farmers to make immediate and informed decisions (Maes and Steppe, 2019). Soil spectroscopy is a technique that uses infrared technologies to swiftly gauge soil properties allowing for more accurate fertilization (Salimi and Boelt, 2019; Mahmud et al., 2023). Today, farmers also have digital allies in the form of Smart Fertilizer Management (SFM) software. These platforms amalgamate data from diverse sources, from soil samples to satellite imagery, and craft fertilizer recommendations tailored to distinct fields (Agrahari et al., 2021). Innovations, such as nanofertilizers, developed via nanotechnology, promise enhanced nutrient efficiency owing to their ability to permeate plant cell walls more effectively than their traditional counterparts (Gomes et al., 2020). The soil microbiome also has potential as a resource. Beneficial microorganisms, such as certain bacteria, can either fix atmospheric nitrogen or render soil phosphorus soluble reducing the dependency on chemical fertilizers and steering agriculture toward more sustainable pathways (Tavarini et al., 2018; González-Guerrero et al., 2023). The combination of technology and biology is further evident in innovations such as *in situ* soil moisture sensors, 3D printed fertilizers tailored to specific crop needs, chlorophyll meters that gauge plant nitrogen levels, and decision support systems (DSS) that assimilate diverse data to guide fertilization decisions (Ahmad and Dar, 2020; Hemming et al., 2020; Gorai et al., 2021). In summary, it is clear that the trajectory of precision fertilization has been marked by rapid advancements. As global challenges surrounding population growth and environmental preservation intensify, these nuanced fertilization techniques stand as pillars for a sustainable agricultural future bridging the gap between high yields and responsible farming.

9.5 Advanced imaging and deep learning for pest management and crop monitoring

Advanced imaging and deep learning technologies have ushered in a revolution in agricultural pest management and crop health assessment. These innovative developments have combined the power of deep learning, including deep neural networks, with a range of imaging modalities, such as hyperspectral, RGB, multispectral, IR, and NIR, to reshape the landscape of agriculture (Figure 3) (Chandel et al., 2021; Liang et al., 2021; Abdullah et al., 2023). This integration has given birth to innovative solutions for pest detection, classification, and localization leading to a significant improvement in agricultural efficiency. Deep learning models have been extensively applied for the detection and diagnosis of plant diseases and pests offering promising results and large potential in image processing and data analysis (Ferentinos, 2018; Kamilaris and Prenafeta-Boldú, 2018; Liu and Wang, 2021), These models have been particularly effective in early pest detection, such as the recognition of insect pests at the larval stage before planting, allowing for precise localization and targeted intervention to minimize chemical usage (Obasekore et al., 2023). This early identification allows for precise localization and targeted intervention minimizing the need for chemical pesticides. Moreover, the accuracy and reliability of pest detection have been significantly enhanced by deep learning technology. Ensemble models based on deep learning have demonstrated the ability to detect anomalies with remarkable performance (Madhavi et al., 2021; Lee et al., 2022). This means that farmers can rely on these models for dependable and timely pest detection reducing the risk of crop damage.

The integration of advanced imaging and deep learning technologies with UAVs has further expanded their impact on agri-pest management. UAVs have become indispensable tools for providing expansive mapping and precise monitoring of agricultural fields. Deep learning-based visual recognition, when paired with UAVs, has enabled the identification of pests and diseases with exceptional accuracy (Tsouros et al., 2019; Ecer et al., 2023). Additionally, the conjunction of deep learning with UAVs has given rise to mobile applications for real-time insect pest detection. These applications consolidate image collection, data preprocessing, and modeling strategies streamlining agricultural practices (Doan, 2022). For example, improved YOLO V3 convolutional neural networks have enabled the high-precision detection of tomato diseases and pests (Liu and Wang, 2020). Similarly, deep learning-based methods for light-trap pest detection have addressed the challenge of varying object numbers and size distributions resulting in improved performance (Teng et al., 2022). A comprehensive survey of recent studies highlights the potential of UAVs in precision agriculture further emphasizing their significance in modern farming practices (Aslan et al., 2022). The integration of advanced imaging modalities with UAVs has contributed to large-scale mapping and enhanced agricultural efficiency. These technologies are not only limited to pest detection but also extend to disease diagnostics and non-destructive methods such as fluorescence imaging.

The development of crop monitoring methods has been significantly influenced by the role of multispectral and hyperspectral imaging in detecting subtle changes in plant health. Hyperspectral imaging, in particular, has proven instrumental in the early detection and classification of plant diseases (Kong et al., 2018; Nagasubramanian et al., 2019). These studies have underscored the potential of hyperspectral imaging as a fast, non-destructive, and reliable technique for disease detection on plant stems. Furthermore, the application of deep learning on hyperspectral images has shown promise in detecting diseases such as potato virus Y in seed potatoes (Polder et al., 2019) and *Aphis gossypii* Glover infection in cotton leaves (Yan et al., 2021). This highlights the profound impact of advanced imaging techniques on disease identification in agriculture. The integration of hyperspectral imaging with remote sensing has opened up new possibilities for detecting and analyzing weed infestations in rice fields (Sulaiman et al., 2022). This application demonstrates the potential of hyperspectral remote sensing imagery in weed detection and analysis contributing to improved crop management practices. Chlorophyll fluorescence imaging has been a valuable tool for capturing the photochemical efficiency of grain sorghum in field settings offering insights into plant photosynthesis and health (Herritt et al., 2020). Additionally, the detection of citrus huanglongbing in Brazilian orchards using hyperspectral aerial images has shown the potential of hyperspectral imaging in identifying and monitoring diseases in agricultural settings (Moriya et al., 2019). These advancements have significantly contributed to our understanding of plant health, disease diagnostics, and crop management ultimately impacting modern agricultural practices and efficiency.

The integration of advanced imaging technologies with unmanned aerial vehicles (UAVs) and AI/ML algorithms has further enhanced their impact on modern agricultural practices. This combination enables rapid and non-invasive characterization of plant health, disease detection, and improved crop management. As documented by Ampatzidis and Partel (2019), this technique offers an early detection system that captures subtle shifts in plant health. Thermal imaging has proven effective in shedding light on plant transpiration dynamics and water-related stress (Shoa et al., 2022). Fluorescence imaging, as described by Valcke (2021), acts as a sentinel for photosynthetic efficiency, flagging potential stressors indicated by fluorescence deviations. Stereoscopic imaging and light detection and ranging (LIDAR) provide spatial insights on plant biomass and terrain (Wiering et al., 2019) facilitating superior drainage systems and optimal layout configurations for crop growth. Simultaneously, X-ray imaging offers a promising modality for meticulous seed quality assessments ensuring that farmers have access to defect-free seeds (de Medeiros et al., 2021). To complete the imaging spectrum, RGB imaging, championed by Mahmud et al. (2023), offers direct monitoring capabilities when integrated with analytical platforms. This simple, yet effective, method provides a clear-cut approach to overseeing growth dynamics and preempting potential challenges. The integration of these technologies with unmanned aerial vehicles and AI/ML algorithms has significantly enhanced their impact on modern farming practices. These advancements not only offer early and precise pest detection but also contribute to large-scale mapping, disease diagnostics, and improved agricultural efficiency.

9.6 Use of AI, ML, IoT, drones, and robotics in vegetable farming

The integration of advanced technologies, including drones, robots, AI, ML, and the IoT, has revolutionized modern agriculture, particularly in the context of precision farming and vegetable cultivation. The adoption of drones in precision farming has seen significant growth, with continuous innovation and selective application of inputs driving this trend (Bai et al., 2022). Similarly, the development of scouting robots equipped with advanced sensors has enabled precise data collection and navigation control contributing to the automation of farming processes (Shamshiri et al., 2018a). Furthermore, the use of AI and ML algorithms in conjunction with IoT technologies has facilitated the implementation of smart agriculture systems leading to improved crop productivity and resource management (Alreshidi, 2019; Adli et al., 2023). In the realm of vegetable farming, precision technologies, cloud computing, and IoT have been harnessed to enhance crop productivity and optimize resource utilization (Kaushik, 2021). The application of AI and ML in smart agriculture has paved the way for the development of IoT-enabled systems for decision making and control resulting in improved crop management and reduced operational costs (Ramakrishnam Raju et al., 2022). Additionally, the integration of AI and IoT technologies has led to the design and implementation of smart hydroponic farming systems further demonstrating the potential for advanced technologies to enhance agricultural practices (Ramakrishnam Raju et al., 2022). The use of robotics in vegetable farming has also been a focal point with robots playing a critical role in preventing future food crises caused by population growth (Li et al., 2022). Furthermore, the integration of AI and IoT technologies has resulted in the development of smart agriculture cloud-based systems enabling remote monitoring and management of agricultural operations (Junaid et al., 2021). The incorporation of intelligent services and cognitive components in IoT architectures has modernized IoT infrastructures allowing for the seamless integration of AI technologies (Valero et al., 2021).

The convergence of AI, ML, and IoT in modern agriculture has not been without challenges. The sheer volume of data generated in smart agriculture systems presents a significant challenge necessitating robust big data analytics and management solutions (Hariri et al., 2019). Additionally, the security and interpretability of AI-based smart agriculture systems have been areas of focus highlighting the need for reliable and secure IoT and AI implementations in agricultural settings (Sabrina et al., 2022; Patel et al., 2023). The integration of drones, robots, AI, ML, and IoT technologies has paved the way for precision farming and vegetable cultivation resulting in enhanced crop productivity, resource management, and operational efficiency. These strides in smart agriculture systems highlight the immense transformative potential of advanced technologies in shaping the agriculture of tomorrow.

10 Sustainability in vegetable cultivation

The agricultural domain is undergoing a transformative shift with the pivot toward more sustainable and organic practices becoming a focal point. In this context, vegetable cultivation has emerged as a linchpin with the onus developing and maintaining vegetable crop health while minimizing ecological impact (Table 1). A burgeoning body of research has underscored this sentiment. One of the seminal works in this sphere is from Otero et al. (2019), who contended that sustainable farming practices are imperative to address the escalating ecological challenges faced by the horticulture sector. This emphasis on sustainability was buttressed by Tahat et al. (2020), who highlighted the virtues of organic growing media. According to their findings, these media not only bolster soil health but also curtail the reliance on depleting non-renewable resources. Adding a layer to this discussion, Rodriguez et al. (2021) compelled the integration of companion plants and cover crops into nurseries. Their research demonstrated that such integrations deter pests and augment soil nutrition consequently

TABLE 1 Sustainable practices in vegetable cultivation.

Sustainability aspect	Practice	Benefits	Common implementations	References
Water conservation	Techniques designed to reduce water usage	Environmental responsibility; cost savings	Rainwater harvesting, drip irrigation	(Bar-Yosef, 2020; Oweis, 2022)
Organic practices	Avoiding synthetic chemicals in favor of natural solutions	Healthier plants; eco-friendly; market demand	Organic fertilizers, biopesticides	(Samada and Tambunan, 2020; Shaji et al., 2021)
Waste recycling	Reusing resources or converting waste into valuable products	Waste reduction; cost efficiency	Composting, mulching	(Zinati, 2005; Sari, 2022)
Renewable energy	Using renewable sources for powering farm operations	Reduce carbon footprint, energy savings; lower operational costs	Solar panels, wind turbines	(Roslan et al., 2018; Grant et al., 2022)
Bio-based growing mediums	Using sustainable, renewable resources as an alternative to traditional growing mediums	Environmental sustainability; improved plant health	Coir, biochar, composted bark, plant growth-promoting rhizobacteria	(López-López and López-Fabal, 2016; Mariotti et al., 2020; Tahiri et al., 2022)
Reduction in energy consumption requirements	Implementing energy-efficient technologies and optimizing resource allocation	Decreased operational costs, reduced environmental impact, enhanced sustainability	Use of solar panels, LED lighting for plant growth, smart sensors for precision agriculture, energy-efficient climate control systems in greenhouses	(Morrow, 2008; Akrami et al., 2020; Goel et al., 2022; Soussi et al., 2022)

reducing the dependence on chemical interventions. Pest management, a perennial concern in horticulture, has been innovatively addressed through sustainable avenues. Nazir et al. (2019) offer an intriguing perspective on this by delving into biological pest control. These studies revealed that beneficial insects, including ladybugs and nematodes, can serve as natural deterrents against harmful pests. Such practices, while ensuring crop health, also safeguard the broader ecological balance. Sustainable materials have emerged as cornerstones of contemporary vegetable cultivation. For example, the proliferation of biodegradable pots, as documented by Cherian et al. (2022), presents a two-pronged advantage. Not only do these pots enhance soil decomposition, but they also significantly reduce plastic waste. Similarly, Li et al. (2021) shine a light on the rise of organic mulches emphasizing their role in moisture retention, weed suppression, and nutrient replenishment. However, the conversation regarding sustainability is incomplete and does not address the role of water. Here, the insights from Bafdal and Dwiratna (2018) stand. They underscored the potential of rainwater collection in nurseries, a practice that leads to marked reductions in water consumption and operational costs. This is complemented by the findings of Redekar et al. (2020) on recycled water systems, which emerge as a paradigm of conservation while ensuring an uncontaminated water supply for crops. Incorporating sustainable practices is not only related to ecological responsibility. Therefore, there is a pressing need to ensure knowledge diffusion for broad-based adoption. Kumar et al. (2018) highlight this very need advocating for comprehensive training programs tailored for farming personnel. The future, as envisioned by Bhandari and Nayama (2020), is one of the syntheses where traditional sustainable practices meld with cutting-edge technologies setting new benchmarks in eco-conscious horticulture. In sum, the trajectory of vegetable management is unequivocal pointing toward an intertwining of environmental stewardship with agricultural progress. The synthesis of research in this domain unequivocally suggests that sustainable vegetable farming is not merely a desirable path but is imperative for the holistic well-being of the global horticulture industry.

11 Potential drawbacks and concerns with new approaches

Like many other sectors, the realm of vegetable farming is undergoing rapid transformation driven by technological advancements. While these innovations offer tremendous potential, they also face a suite of challenges. This review attempts to shed light on the multifaceted concerns that these advancements have brought to the fore.

Environmental concerns: *Resource overconsumption*: Modern systems, in their bid to maximize outputs, often demand high energy or water inputs. Although they might bolster productivity, they can inadvertently strain our already dwindling resources (Ghasemi-Mobtaker et al., 2020). *Waste generation* further aggravates environmental concerns. The infusion of non-biodegradable components into many new technologies escalates waste production especially when recycling options are scarce (Kibria et al., 2023).

Biological and ecological concerns: The realm of biology is immune to these concerns. *Overreliance* on a limited pool of genetically engineered plants threatens the biodiversity. This homogeneity can make crops vulnerable to diseases, thereby weakening the resilience of ecosystems (Caradus, 2022). Adding to ecological woes is the *chemical residue* left behind by synthetic growth promoters and pest control agents. These residues can harm beneficial organisms and have lasting repercussions for environmental health (Mateos Fernández et al., 2022).

Economic and social concerns: From economic standpoint, the risk of *market dependency* is large. Certain cutting-edge techniques might hinge on patented inputs, providing disproportionate market power to a handful of corporations. This scenario can spawn dependency and lead to monopolistic behaviors that impact pricing (Hussain et al., 2020). *Equity concerns* are paramount. The disparity between large-scale, affluent nurseries and their smaller, resource-constrained counterparts could widen because the former might have exclusive access to expensive high-tech solutions (Talukder et al., 2020).

Technological concerns: Technological integration, while impressive, presents its own set of challenges. The complex nature of new systems can compromise *reliability*. Malfunctions in such intricate setups can result in substantial losses (Mistry et al., 2020). Additionally, an *over-dependency* on technology can lead to the erosion of traditional skills. In scenarios where technology falters or is accessible, skill attrition can have significant consequences.

Although the allure of technological and innovative strides is undeniable, a circumspect approach is essential. It is vital to temper enthusiasm with caution to ensure that the broader implications of each advancement are thoroughly vetted. The cited studies offer deeper insights and provide a foundation for anyone seeking a profound understanding of these challenges.

12 Future prospects

The evolution of advanced vegetable cultivation practices has thus far been nothing short of being remarkable. Over the past decade, the integration of technology with traditional practices has sculpted the landscape of immense potential. While predicting the future with pinpoint accuracy is ambitious, the present trends and technological underpinnings offer illuminating insights.

12.1 Prediction of future advances in vegetable farming

Genomic insights: The roadmap of genomic research points to a future where the genetic blueprint of plants holds the key to transformative practices. The prospect of plant varieties tailored to regional idiosyncrasies, individual crop specifications, or particular soil types is becoming increasingly tangible (Bhowmik et al., 2021).

IoT integration: IoT is merely a buzzword, the backbone of the next industrial revolution. In the context of vegetable farming, imagine a world where real-time monitoring systems assessing variables, such as soil moisture or light levels—feed data that automatically modulate crop environments (Naik et al., 2021).

VR and AR in training: VR and AR revolutionize training paradigms. From in-depth anatomical explorations of plant structures to real-time disease diagnostics, these technologies can facilitate immersive learning experiences and transform novices into experts (Yousif, 2022).

Decentralized AI-driven decisions: The shift from centralized to decentralized (or edge) computing can redefine the response times in crop management. With decisions anchored at the device level, the latency in system responses can plummet making management more agile (Slob and Hurst, 2022).

Circular economy in vegetable farming: The call for sustainability is answered with the circular economy model. Its ethos of resource conservation, emphasizing the loop of reuse, recycling, and upcycling, will steer vegetable cultivation toward unprecedented sustainability milestones (Zarbà et al., 2019).

Personalized plant care: The paradigm of personalized care is confined to human medicine. Precision horticulture, built on the premise of customizing care to individual plant needs, can elevate plant health and optimize resource allocation (López et al., 2009; Atreya et al., 2019).

Essentially, the future trajectory of vegetable farming sparkles with a blend of traditional knowledge and avant-garde innovations, amplifying sustainability, and efficiency. However, the path is speckled with challenges in navigating ethical conundrums discerning economic implications and ensuring the reliability of emerging technologies. Progression in this sector will hinge on an equilibrium between empirical research and informed judgment paving the way for a thriving nexus between flourishing plants and evolved practices.

12.2 The role of research and development in orchestrating vegetable cultivation renaissance

In the vast tapestry of industrial evolution, research and development (R&D) has emerged as a silent weaver stitching together the complex interplay of science, technology, and market demand. Vegetable farming, a field where nature meets nurture, finds R&D an ally invaluable. This is a deeper exploration of the transformative role of R&D.

1. **Bridging practical and theoretical knowledge:** The dynamism of R&D transforms theoretical paradigms into practical solutions. It acts as a conduit, ensuring that laboratory breakthroughs are confined to scholarly papers, but find resonance in the soil and seeds of nurseries.
2. **Tailored solutions for specific challenges:** One-size-fits-all is an anachronism in modern crop cultivation practices. R&D, with its deep dive into regional idiosyncrasies, crafts solutions that address unique challenges, such as the saline soils of coastal regions or drought-prone terrains of the hinterlands.
3. **Integration of advanced technologies:** R&D is the crucible in which the technology mettle was tested: the evolution of drone-based monitoring systems, IoT-integrated irrigation models, and AI-driven pest prediction matrices, and their R&D that catalyze their journey from prototypes to practical solutions.
4. **Sustainable and organic innovations:** As consumers' palates become discerning, prioritizing organic over synthetic, R&D shoulders the responsibility of developing eco-friendly yet effective vegetable farming strategies. This entails research on sustainable pesticides, eco-friendly fertilizers, and holistic farming practices.
5. **Improved seed varieties:** At genetic frontier, R&D deciphers the code of life leading to the genesis of seed varieties that can weather adversities, resist diseases, and flourish even in challenging environments.
6. **Skill development and capacity building:** Beyond tangible aspects, R&D has an educational facet. It nurtures talent, equipping the custodians of nurseries with skills that marry traditions with technology, ensuring the industry's vibrancy and relevance.
7. **Feedback loop creation:** R&D is not a monolog; it thrives on dialog. The feedback loop it establishes ensures that research is not conducted in silos but is continually refined based on ground-level insights and real-world challenges.

To encapsulate, R&D is a compass that ensures that vegetable farming does not meander but marches forward with purpose and clarity. It is the harbinger of a future where vegetable cultivation farm are not spaces of crop cultivation but cradles innovation. For the industry to flourish, its stakeholders must not just apply R&D from the sidelines, but must actively champion and invest in it, recognizing its unparalleled role in shaping tomorrow.

13 Conclusion

In the present milieu, vegetable cultivation practices are on the brink of significant metamorphosis. This comprehensive review intricately traversed the various layers of this transformation ranging from the delicate intricacies of seed choice to wider frameworks centered on sustainability and impending advancements. The forefront of our discourse is several pivotal insights. The fusion of genomics with advanced evaluation strategies suggests a promising path for cultivating both resilient and prolific crops. There has been a discernible shift in foundational vegetable farming practices highlighted by the rise of soilless cultures and advanced environmental modulating techniques. Water and nutrition management now underlines precision and sustainability reflecting a sector-wide inclination toward optimal resource stewardship. Current trends in pest management and growth regulation have revealed a diminishing reliance on chemical means paralleled by inventive strategies that promise greater agricultural output. Furthermore, the merger of traditional farming practices with emerging technologies, such as drones and AI, sketches the portrait of a rapidly transforming vegetable cultivation landscape. The move toward environmentally responsible measures complements an invigorating future painted with the strokes of genomics, the IoT, and AI. However, anchoring these progressive deliberations is an unwavering emphasis on solid research and development foundations. Beyond these specific insights, a more expansive theme becomes evident: the non-negotiable need for relentless research and fluid strategy. In a realm as dynamic as vegetable farming, where challenges such as ever-evolving pests and shifting climatic norms are given, the crux lies in persistent research and adaptability. By navigating new obstacles to embracing technological breakthroughs, prioritizing sustainability, and maximizing yield, these two facets stand out as central tenets. In conclusion, vegetable cultivation practices reflect a delicate balance between age-old practices and modern paradigms. While the journey is rife with challenges, these are set against a canvas teeming with potential. For those vested in this domain, the mandate is clear: invest in learning, celebrate flexibility, and gear up for a horizon rich in both trials and triumphs, which are all crucial to ensuring global food stability.

Author contributions

NA: Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. BZ: Conceptualization, Writing – original draft, Writing – review & editing, Resources, Validation. LD: Formal analysis, Investigation, Writing – review & editing. BB: Software, Visualization, Writing – review & editing. JL: Conceptualization, Formal analysis, Writing – review & editing. SC: Investigation, Methodology, Writing – review & editing. ZC: Formal analysis, Investigation, Writing – review & editing. IJ: Investigation, Visualization, Writing – review & editing. AT: Investigation, Validation, Writing – review & editing. MG: Investigation, Resources, Writing – review & editing. FH: Formal analysis, Investigation, Writing – review & editing. PT: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

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

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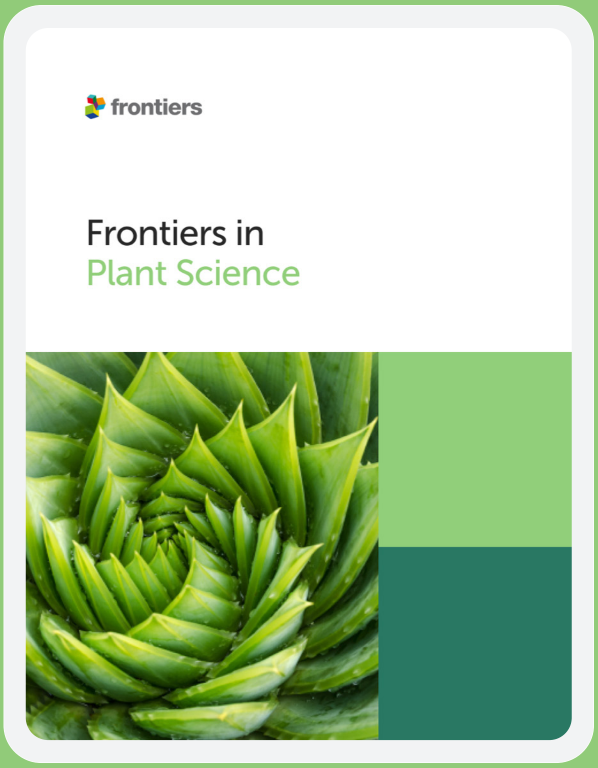

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