Agricultural Water Management 285 (2023) 108365

Contents lists available at ScienceDirect

Agricultural Water Management

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

Improving water use efficiency in vertical farming: Effects of growing systems, far-red radiation and planting density on lettuce cultivation

Laura Carotti [a], Alessandro Pistillo [a], Ilaria Zauli [a], Davide Meneghello [b], Michael Martin [c], Giuseppina Pennisi [a,*], Giorgio Gianquinto [a], Francesco Orsini [a]

[a] Alma Mater Studiorum – University of Bologna, DISTAL – Department of Agricultural and Food Science, Viale Giuseppe Fanin 44, 40127, Bologna, Italy
[b] FRM srl, Piazza Manifattura 1, 38068 Rovereto, Italy
[c] IVL Swedish Environmental Research Institute, Valhallavägen 81, 114 28 Stockholm, Sweden

ARTICLE INFO

Handling Editor - R. Thompson

Keywords:
Plant factories with artificial lighting (PFALs)
Aeroponics
Ebb-and-flow
Closed-loop systems
Circularity

ABSTRACT

Vertical farms (VFs) are innovative urban production facilities consisting of multi-level indoor systems equipped with artificial lighting in which all the environmental conditions are controlled independently from the external climate. VFs are generally provided with a closed loop fertigation system to optimize the use of water and nutrients. The objective of this study, performed within an experimental VF at the University of Bologna, was to quantify the water use efficiency (WUE, ratio between plant fresh weight and the volume of water used) for a lettuce (*Lactuca sativa* L.) growth cycle obtained in two different growing systems: an ebb-and-flow substrate culture and a high pressure aeroponic system. Considering the total water consumed (water used for irrigation and climate management), WUE of ebb-and-flow and aeroponics was 28.1 and 52.9 g L^{-1} H_2O, respectively. During the growing cycle, the contribution generated by the recovery of internal air moisture from the heating, ventilation and air conditioning (HVAC) system, was quantified. Indeed, by recovering water from the dehumidifier, water use decreases dramatically (by 67 %), while WUE increased by 206 %. Further improvement of WUE in the ebb-and-flow system was obtained through ameliorated crop management strategies, in particular, by increasing planting densities (e.g., 153, 270 and 733 plants m^{-2}) and by optimizing the light spectrum used for plant growth (e.g., adjusting the amount of far-red radiation in the spectrum). Strategies for efficient use of water in high-tech urban indoor growing systems are therefore proposed.

1. Introduction

Producing more food while using fewer natural resources is one of the biggest challenges in the climate change adaptation processes (Wiebe et al., 2019; Malhi et al., 2021). Agricultural activities have a great impact on freshwater availability, accounting for about 70 % of freshwater withdrawals (FAO, 2014). Moreover, according to climate prediction models, rising temperatures in several countries will result in severe water shortages (Agovino et al., 2019). Considering these scenarios, the implementation of innovative technologies enabling to reduce water use, while maintaining or increasing the yield, is a priority for the agricultural sector (Incrocci et al., 2020; Michelon et al., 2021).

Among these innovative technologies, vertical farms (VFs) are indoor growing structures completely insulated from the outside environment, generally located in urban and peri-urban environments, where plants are normally cultivated in multi-layer growing systems using high planting density (Kozai and Niu, 2016; Avgoustaki and Xydis, 2021). In this plant production system, all the environmental parameters may be precisely controlled guaranteeing a stable year-round production, independently from outside conditions and season (Orsini et al., 2020; van Delden et al., 2021; Kalantari et al., 2018).

Vertical farming is highlighted as one of the horticultural strategies that can lead to a great resource use efficiency (Orsini et al., 2020; Kozai and Niu, 2016), defined as the amount of resource fixed or utilized by plants compared to the amount of resource supplied to the system (Kozai and Niu, 2016). When compared to greenhouses, VFs have demonstrated to reduce the water use by 28–95 %, according to the geographical area in which the growing facilities are located and the greenhouse technologies adopted (Graamans et al., 2018). Defining the water use efficiency (WUE) as the grams of fresh weight (FW) produced per liter of water used in the system, literature reports that lettuce production in VFs can reach values as high as 80 g FW L^{-1} H_2O,

---

* Corresponding author.
E-mail address: giuseppina.pennisi@unibo.it (G. Pennisi).

https://doi.org/10.1016/j.agwat.2023.108365
Received 11 January 2023; Received in revised form 10 May 2023; Accepted 11 May 2023
Available online 23 May 2023
0378-3774/© 2023 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

significantly greater than those obtained in greenhouse and open field for the same crop (up to 60 and 20 g FW L^{-1} H_2O, respectively) (Orsini et al., 2020). These great performances are mainly due to the use of modern soilless growing systems, where irrigation is usually operated on a closed loop (Al-Kodmany, 2018; Orsini et al., 2020).

Soilless systems are generally classified into substrate culture and hydroponic culture (Gruda, 2020). In both systems, water supplies nutrients to the roots in the form of a nutrient solution. In substrate culture, plants are commonly hosted in pots with an inert substrate and nutrient solution is continuously or periodically distributed, as in the case of the ebb-and-flow system. In the hydroponic culture plant roots are directly in contact with the nutrient solution without any solid material (Gruda, 2020; Eldridge et al., 2020). There are different types of hydroponic cultures, which differ mainly in the way the nutrient solution is circulated. Some examples are the deep-water culture (DWC), where the roots are constantly submerged in the nutrient solution, and the aeroponic cultivation, where plant roots are periodically sprayed with a fine mist of nutrient solution (Lakhiar et al., 2018; Eldridge et al., 2020). Thanks to a more precise management of the root zone, the aeroponic system is usually claimed to better performing, although limited literature has to date addressed the comparison with other more widely used soilless systems for vertical farming, such as the ebb-and-flow (Eldridge et al., 2020; Orsini et al., 2020).

Independently from the system typology, the adoption of closed loops allows for the re-use of water and nutrients drained from the growing layer. After checking its pH and EC and restoring nutrients' level to set point values, the nutrient solution may be used again to water the plants (Avgoustaki and Xydis, 2021). In this way, WUE is maximized, and both water and nutrients that have not been used by plants may be recycled (Putra and Yuliando, 2015; Langenfeld et al., 2022), also reducing impacts on eutrophication processes (Rufí-Salís et al., 2020).

Beside the specific characteristics of the cultivation system, WUE can be improved through an adequate microclimate management and the ability to reuse water collected from the heating, ventilation and air conditioning (HVAC) system (Katsoulas et al., 2015). Due to plants transpiration, air moisture is high within VFs, and through the condensation process it is possible to recover water that can be reused for irrigation, thus creating a close water cycle (Jurga et al., 2023; Soussi et al., 2022). Several theoretical models have estimated the contribution of water recovered from the HVAC system in both greenhouses (Lovichit et al., 2007; Yildiz and Stombaugh, 2006) and vertical farms (Pacak et al., 2020), and given the interest in this type of analysis, it is estimated that the number of models will increase in the coming years. In contrast, the number of studies that empirically quantify the contribution of water recovered from the HVAC system is still limited.

The advanced cultivation techniques and detailed cultivation protocols tailored to the crop needs are also recognized as having a key role in increasing WUE (Orsini et al., 2023; Tavan et al., 2021). Controlled growing conditions that are applied in a vertical farm can in fact increase yield or decrease the required water contribution, as for instance in the case of CO_2 concentration (Langenfeld et al., 2022) and light characteristics.

For what concerns the light, many studies have examined the effects of light quality on crops' growth and quality (Pennisi et al., 2019; Appolloni et al., 2021). Particularly for growth, the major research effort has been focused on the effect of modulation of red (600–700 nm) and blue (400−500 nm) wavelengths, which match the absorption peaks of the principal pigments (chlorophyll a and b) involved in light absorption for photosynthesis (Son and Oh, 2015). Far-red radiation (700–780 nm), on the other hand, has traditionally been excluded from the definition of photosynthetically active radiation (PAR) since its contribution to plants' photosynthesis has always been considered negligible when applied alone (McCree, 1971). However, recent studies showed that in addition to morphological responses (Demotes-Mainard et al., 2016; Zou et al., 2019), far-red radiation can positively boost photosynthesis (Zhen and Bugbee, 2020), when applied in combination with shorter wavelengths (e.g., red and blue). Since LED lights allow for a fine-tuning of the spectrum emitted by the lamps (Massa et al., 2008), precisely managing the far-red radiation, and in particular its intensity, can be a strategy to increase yield.

The adoption of an optimal planting density is an important parameter in VFs where higher densities as compared to other cultivation systems (e.g., greenhouses or open field) are usually used to improve yield per unit of area (Jin et al., 2022; Orsini et al., 2020). In the case of lettuce cultivation, several studies carried out in VFs or growth chambers use a planting density of around 140 plants m^{-2} on average (Jin et al., 2022). Considering that environmental factors can potentially be non-limiting, testing higher planting densities, in combination with precise management of cultivation techniques, can lead to an increase in resource use efficiency, decreasing the crop water use and increasing the light capture from the canopy (Ramin Shamshiri et al., 2018).

To the best of our knowledge, there is very little work comparing water use in aeroponics and ebb-and-flow using the same environmental conditions in a vertical farm with artificial light. Accordingly, the objectives of this work were: 1) evaluating water use efficiency in a vertical farm for lettuce production, considering both the adoption of closed loop soilless systems and the contribution from the water recovery from the HVAC system; 2) comparatively assess the water use efficiency performances and the lettuce growth in a vertical farm either adopting an ebb-and-flow or an aeroponic system; 3) explore and describe the effect of different cultivation techniques on water use efficiency in the ebb-and-flow system, such as the specific management of far-red radiation in the light spectrum and the use of increasing planting density.

2. Materials and methods

2.1. Experimental facility and growing conditions

The trials have been conducted within AlmaVFarm, an experimental vertical farm located in the Department of Agricultural and Food Sciences at the University of Bologna. The facility is composed by an isothermal cell (45 m^2), containing two growing system typologies, respectively an ebb-and-flow and a high pressure aeroponic (Frm srl, Rovereto, TN, Italy), organized in 22 cultivation sectors (10 ebb-and-flow + 12 aeroponics), each composed by 3 stacked levels. Each level consists of a cultivation tray with a surface of 0.53 m^2. Additionally, 3 more aeroponic sectors are dedicated to germination, with 5 stacked growing layers. Additional details on the spatial arrangement of the facility are given in Fig. 1 A and B.

The facility is equipped with a heating, ventilation and air conditioning (HVAC) system (Monti&C srl, Borgo a Buggiano, PT, Italy) for cooling, heating and ventilating, and with a dehumidifier consisting of a duct system for supply and return of treated air that recovers the water transpired by the plants. The ventilation system avoids air stratification, by recirculating internal air. An hourly exchange rate with the external atmosphere was set at 0.03 m^3 m^3 h^{-1}. During the trials, air temperature and humidity of the vertical farm were set at 24/21 ± 1 °C day/night and 70/75 ± 10 % day/night respectively, and additional CO_2 was supplied in order to maintain a constant concentration of 850 ppm.

The growing systems consist of closed-loop water cycles, with the drained water returning to a water tank, where fertigator (NidoPro®, LogicSun, Cattolica, RN, Italy) checks (96-time day^{-1}), and if necessary, corrects pH and EC parameters before returning the nutrient solution to the water pump and then to the system. The same nutrient solution formulation (EC=2.3 dS m^{-1} ± 0.2, pH=6.5 ± 0.2) was used for each experiment, with the following composition: N-NO_3: 14 mM; N-NH_4: 4.4 mM; P: 1.0 mM; K: 5.0 mM; S: 2.0 mM; Ca: 5.2 mM; Mg: 1.2 mM; Fe: 17.9 μM, Cu: 2.0 μM, Zn: 3.8 μM, B: 11.6 μM, Mn: 18.2 μM, Mo: 0.5 μM.

In the aeroponic sectors, where each tray contained 4 nozzles (diameter of the nozzle orifice = 0.2 mm), the pressured (70 bar) nutrient solution was nebulized to the plants' roots every 15 min for 90 s during the whole growing cycle. Considering the pressure and the

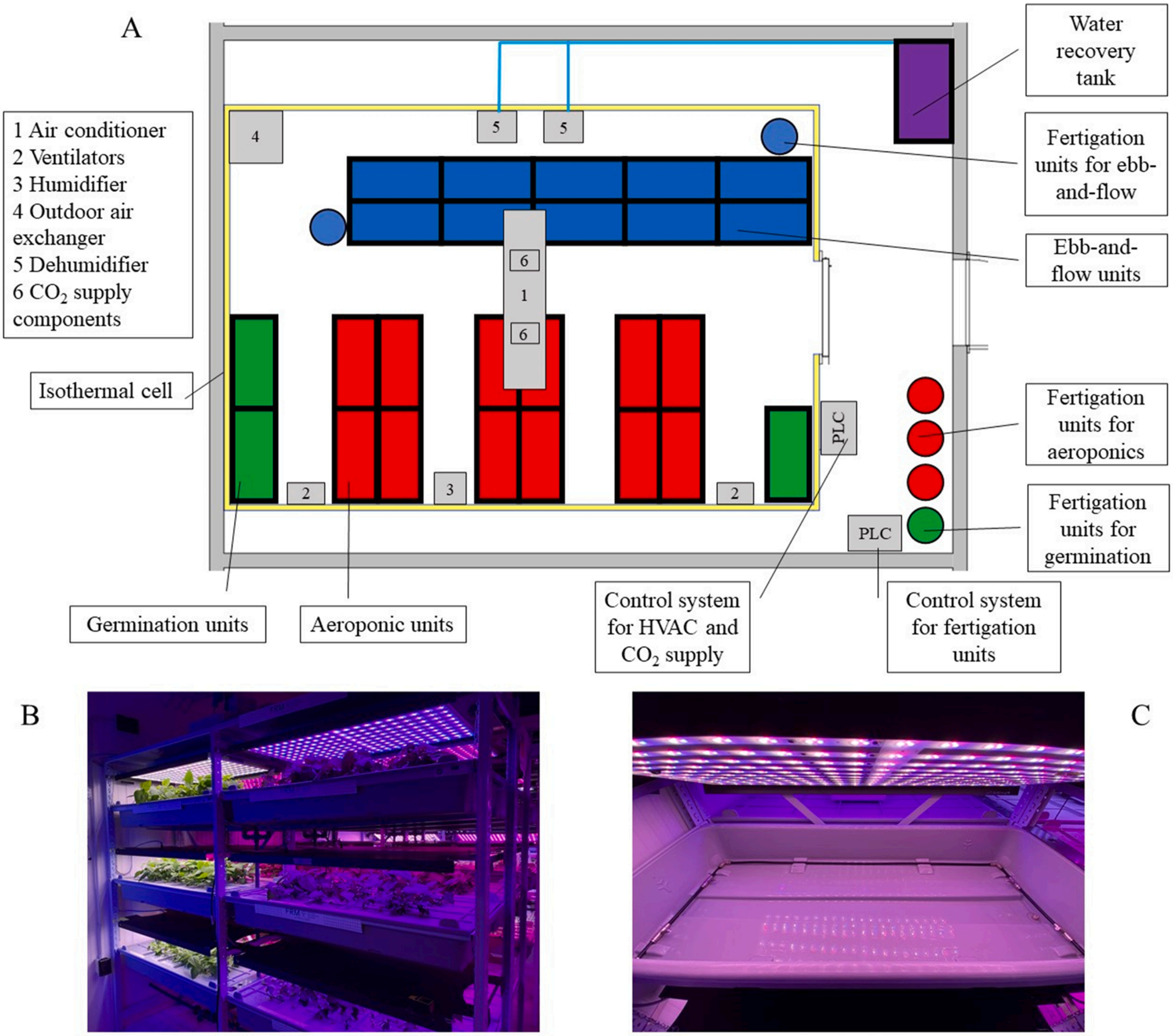

Fig. 1. A) Aerial view of the experimental vertical farm. Green rectangles are the aeroponic sectors dedicated to the germination, red rectangles the aeroponic sectors dedicated to plants' growth and blue rectangles the ebb-and-flow sectors dedicated to plants' growth. In grey, the HVAC (heating, ventilation and air conditioning) system components: 1) air conditioner; 2) ventilator 3) humidifier; 4) outdoor air exchanger; 5) dehumidifier and 6) CO2 supply components. In light blue, pipes to move water recovered from dehumidifier to water recovery tank (in purple). Circles represent the fertigation units (in blue for ebb-and-flow, in red for aeroponics, in green for germination sectors). B) Aeroponic units. C) Detail of nozzles disposition in the aeroponic trays.

diameter of the nozzle orifice, droplets between 10 and 30 μm were generated (nozzles are visible in Fig. 1 C). In the ebb-and-flow sectors, the nutrient solution was circulated once a day for 30 min for the first two weeks of each growing cycle (from 1 to 14 DAT), and twice a day for 20 min each for the last two weeks (from 15 DAT to end of the cycle). A summary of the environmental parameters and the nutrient solution is given in Table 1.

Three different experiments were conducted, as reported in the paragraphs below. Table 2 shows a summary of the three experiments performed.

2.2. *Experiment 1 (exp. 1): lettuce growth and water use*

The first experiment started on February 14, 2022 and ended on March 29, 2022. Lettuce seeds (*Lactuca sativa* L. var. Canasta) were sown in plastic net-pots containing plugs of a biodegradable polymer

Table 1
Summary of the environmental parameters kept constant (temperature (°C), relative humidity (%), photoperiod (h day^{-1}), pH, electrical conductivity (dS m^{-1}) and CO$_2$ level (ppm)) during the experimental period.

Temperature (°C)	Relative humidity (%)	Photoperiod (h d^{-1})	pH	Electrical conductivity (dS m^{-1})	CO$_2$ level (ppm)
24/21 ± 1 day/night	70/75 ± 10 day/night	16	6.5 ± 0.2	2.3 ± 0.2	850

Table 2
Aim of the experiment, type of growing system adopted (ebb-and-flow or aeroponics), planting density (plants m^{-2}), and light spectrum used for experiment 1, 2 and 3. For light spectrum, RB_3 refers to a spectrum composed by red and blue radiation, in a specific ratio of 3:1. RB_3-30, RB_3-50 and RB_3-70 refer to an RB_3 spectrum where 30, 50 and 70 μmol m^{-2} s^{-1} of red and blue radiation have been replaced by the same amount of far-red radiation.

Experiment	Aim	Growing system	Planting density (plants m^{-2})	Light spectrum
1	Lettuce growth and water use in AlmaVFarm	Ebb-and-flow	153	RB_3
		Aeroponics	131	RB_3
2	Optimization of light spectrum	Ebb-and-flow	153	Experimental: RB_3, RB_3-30, RB_3-50 and RB_3-70
3	Optimization of planting density	Ebb-and-flow	Experimental: 153, 270 and 733	RB_3

(GROWFOAM®, Foamplant BV, Groningen, The Netherlands) and placed in the aeroponic system dedicated to germination. Light was provided by red and blue LED lighting (Flytech srl, Belluno, Italy), in a specific ratio of 3:1 (RB_3; Pennisi et al., 2019), with a PPFD of 200 μmol m^{-2} s^{-1} and a photoperiod of 16 h d^{-1}. At the second true leaf stage, 14 days after sowing (DAS), plants were moved into their final growing systems. For the ebb-and-flow system, plants were transplanted into 6 × 6×7 cm (0.20 L) plastic pots containing peat, and then placed into the stacked levels with a planting density of 153 plants m^{-2}, occupying a total surface of 11.4 m^2. The transplanting into the ebb-and-flow system took place in the vertical farm itself: the plants, altogether with the plugs, were gently removed from the net-pots and transplanted into the plastic pots filled with peat, avoiding any potential damage to the roots. For the aeroponic system, the plants within the plastic net-pots were simply placed into the trays with a planting density of 131 plant m^{-2}, occupying a total surface of 15.0 m^2. Although we acknowledge that the different planting density could have altered the amount of lighting received by plants in the two systems, as well as their final yield, the aim of the research was to comparatively assess performances in a commercially productive setting, e.g., adopting commonly used items (net-pots, pots, trays) in each of the two systems tested. Nutrient solution, fertigation cycles and environmental conditions are described in Section 2.1. In the aeroponic system, light conditions were the ones applied during germination (spectrum RB_3, PPFD of 200 μmol m^{-2} s^{-1}, photoperiod of 16 h d^{-1}), while in the ebb-and-flow system, different spectra were applied, as explained in Section 2.3. For the analysis of growth and physiological data, only plants grown under RB_3 spectrum were considered, corresponding to 12 sectors for the aeroponics and 2 sectors for the ebb-and-flow system.

Plants were harvested 30 days after transplanting (DAT). Measures were carried out on 5 plants per level, for a total of 15 plants per sector. Leaf fresh weight (LFW) and leaf dry weight (LDW) (obtained after drying the leaves for 72 h at 48 °C) were measured with a precision balance. Yield was calculated by multiplying the LFW by the number of plants per m^2, and the dry matter content (DMC) by the ratio between LDW and LFW. Stomatal conductance was assessed using a leaf porometer (AP4, Delta-T Devices, Cambridge, United Kingdom) taking three measurements for each of the two most developed leaves. Leaf greenness was estimated using a hand-held leaf chlorophyll meter (SPAD-502, Konica Minolta, Chiyoda, Tokyo, Japan) by performing three measurements for each of the two most developed leaves. From the transplanting until the final harvest, all water consumptions associated with the various components of the systems were monitored. The water entering the system was monitored daily by means of flowmeters at the general water input and at the pumps of the ebb-and-flow and aeroponic systems. In addition, specifically for the exp. 1, water recovered from the HVAC system, which under normal conditions returns water to the fertigation units, was diverted onto another tank, in order to be quantified.

2.3. Experiment 2 (exp. 2): optimization of the light spectrum in the ebb-and-flow system

Experiment 2 was carried out simultaneously with exp. 1 (from February 14, 2022 to March 28, 2022) and involved 4 ebb-and-flow cultivation sectors. Germination phase was the same as that described in exp. 1. At transplanting (14 DAS), 4 lighting spectra were applied: the RB_3 spectrum was used as a control, and 30, 50 or 70 μmol m^{-2} s^{-1} (namely RB_3-30, RB_3-50 and RB_3-70, respectively) of red and blue radiation were substituted by the same amount of far-red radiation (FR, peak at 729 nm). In all light treatments the ratio between red and blue radiation was maintained constant at 3:1 (RB_3), as also the photon flux density (set at 200 μmol m^{-2} s^{-1}) and the photoperiod (16 h d^{-1}). Specific characteristics of the lighting regimes, such as the far-red fraction (Kusuma and Bugbee, 2021), are given in Table 3. Nutrient solution, fertigation cycles and environmental conditions are reported in **Section 2.1** .

Plants were harvested at 29 DAT. Measures were carried out on 5 plants per treatment, for a total of 15 plants per sector. LFW and LDW, stomatal conductance and leaf greenness were measured as reported for exp. 1. Leaf area was assessed through the open software and app Easy Leaf Area, developed by University of California (USA). DMC was calculated as reported for exp. 1 and leaf area index (LAI) was calculated by dividing the total leaf area by the unit of ground.

2.4. Experiment 3 (exp. 3): optimization of planting density in ebb-and-flow system

The third experiment started on June 6, 2022 and ended on July 22, 2022. Lettuce (*Lactuca sativa* L. var. Canasta) seeds were sown into polystyrene containers filled with peat where they remained for 14 days, inside a growth chamber. Temperature was set at 20/18 °C day/night and HPS lamps with an intensity of 200 μmol $m^{-2}s^{-1}$ and a photoperiod of 16 h d^{-1} were used. After 14 DAS, with the second true leaf well developed, plants were moved into the vertical farm and transplanted in 3 ebb-and-flow cultivation sectors, where three different planting densities were applied, namely 153, 270 and 733 plants m^{-2}. For the first two planting densities, lettuces have been transplanted in peat-filled plastic pots (6 ×6×7 cm; 0.20 L), while for the highest density (733 plants m^{-2}) plants have been kept in the same polystyrene containers used for germination. Although, it is recognized that the different containers may have altered plants root system and the overall plant development, it was chosen to adopt technical solutions that would normally be pursued by a grower (e.g., when using lower density, increasing pot size). An RB_3 spectrum, with 200 μmol m^{-2} s^{-1} and a photoperiod of 16 h d^{-1} were applied for the entire growth cycle in all the sectors. With the exclusion of the germination period, nutrient solution, fertigation cycles and environmental conditions were the same as those described in **Section 2.1** . Harvesting was performed at 20 and 33 DAT (measuring 12 plants per level, for a total of 36 plants per treatment). LFW, LDW, yield and DMC were evaluated as described in exp. 1. For the monitoring of water consumption, the same procedure described for exp. 1 was used.

2.5. Water use efficiency analysis

The ratio between leaf fresh weight and water used in response to the different experimental treatments was used to evaluate different water

Table 3
Spectral characteristics (red and blue and far-red radiation intensity; μmol m^{-2} s^{-1}), total photosynthetic photon flux density (PPFD; μmol m^{-2} s^{-1}, 400–700 nm), total photon flux density (PFD; μmol m^{-2} s^{-1}, 400–780 nm), far-red fraction (FR/R+FR) and percentage of far-red radiation in the spectrum for the four different light treatments: RB_3, RB_3-30, RB_3-50 and RB_3-70.

Light treatments	RB_3 (μmol m^{-2} s^{-1})	FR (μmol m^{-2} s^{-1})	Total PPFD (μmol m^{-2} s^{-1}, 400–700 nm)	Total PFD (μmol m^{-2} s^{-1}, 400–780 nm)	FR fraction (FR/R+FR)	FR percentage (%)
RB_3	200	0	200	200	0	0
RB_3-30	170	30	170	200	0.2	15
RB_3-50	150	50	150	200	0.3	25
RB_3-70	130	70	130	200	0.4	35

use efficiency scenarios. For exp. 1, depending on the scenario considered for the analysis, water use (WU) was calculated as the water consumed for the irrigation (W_i):

$$WU = W_i \tag{1}$$

or W_i plus the water used for the HVAC system and farm operations (W_c) (2):

$$WU_t = W_i + W_c \tag{2}$$

For the scenario in which the water recovered by the dehumidifier was considered, the WU (WU_f) was calculated by subtracting the water recovered from the HVAC system (W_r) from WU_t (3):

$$WU_f = WU_t - W_r \tag{3}$$

For exp. 2 and 3, water use efficiency variations were only determined by change in fresh weight. Indeed, water use was not measured individually for the different treatments. For these experiments, WU_f was used.

2.6. Statistical analysis

Data were analyzed by a one-way ANOVA analysis, using SPSS statistic. Significant differences between light treatments were tested by Tukey test at 95 % confidence.

3. Results and discussion

3.1. Water use in AlmaVFarm

In the experimental vertical farm analyzed in this study, ebb-and-flow and high pressure aeroponic systems were considered, both equipped with close-loop water recirculation systems. For the lettuce growing cycle carried out simultaneously in the ebb-and-flow and in aeroponics (exp. 1), 1870 L of water were used (Table 4). The liters used were allocated between the various components as follow: 767 L for irrigation in the ebb-and-flow system (67.0 L m^{-2}), 504 L for irrigation in the aeroponic system (33.5 L m^{-2}) and 599 L used both from the HVAC system and by operators for daily cleaning operations. In the latter case, the total amount of water used was divided by the total surface of the facility, resulting in 13.3 L m^{-2}.

Table 4
Water use (L and L m^{-2}) for the various components of the vertical farm during one lettuce growth cycle. Liters per m^2 were calculated using the growing area of ebb-and-flow and aeroponic systems, respectively. The consumption of the HVAC (heating, ventilation and air conditioning) system was divided by the total surface of the growing facility.

Water use in AlmaVFarm	L	L m^{-2}
Irrigation in the ebb-and-flow system	767	67.0
Irrigation in the aeroponic system	504	33.5
HVAC system (humidification processes) + management operations	599	13.3
Total water use	1870	
Dehumidifier (water recovered from the internal environment)	1263	

Water used for irrigation and by the HVAC system and operations accounted for 68 % and 32 % of the total (Table 4), respectively. Usually, moving the water utilization efficiency assessment from a growth chamber to a larger facility, water use increases mainly due to the amount of water employed for cleaning and sanitizing operations. Indeed, Ohyama et al. (2020) reported using 70 % of total water use for operations such as trays and equipment cleaning. This percentage of course, can widely vary according to the type of operations that are carried out in the facility. In our case (the research was performed in an experimental vertical farm, with cleaning operations performed inside the farm), water used for cleaning operations was the only amount of water not reused by the system, thus accounting for the unique water loss. Indeed, in our experiments, 67 % of water used as input was recovered from the HVAC system, accounting for 1263 L (Table 4). There are different ways to collect water from the internal environment. In the study by Pacak et al. (2020), the potential of water recovery from the exhausted air of the growth chamber using the cross-flow plate heat exchanger was checked by the application of a mathematical model. Results showed that the water recovered was able to cover the water requirements for irrigation, when the model was applied to moderate climates.

Beside the theoretical model, the potential of water recover was also tested by Ohyama et al. (2000) in a study performed in a growth chamber, during a 15-day growth cycle of sweet potato plants. Their data showed that up to 76 % of the water supplied can be recovered for further irrigation through air dehumidification. The higher value obtained can be the result of higher temperature (30 °C) and relative humidity (80 %) adopted in their experiment, which may have led to different stomatal activity and thus a different level of water transpired by plants and different amount of water used. In addition, the type of facility and of HVAC system, as well as its efficiency, can affect the amount of water that it is possible to recover from the dehumidification process (Algarni et al., 2018).

Table 5
Yield (kg FW m^{-2}), plant fresh weight (g $plant^{-1}$), dry matter content (%), stomatal conductance (mmol m^{-2} s^{-1}) and leaf greenness (SPAD units) for plants grown in ebb-and-flow and aeroponics. Data ± standard error (n = 15) are reported. Different letters indicate significant differences according to the ANOVA test and the Tukey post-hoc test for mean separation with $p < 0.05$.

Growing system	Yield (kg FW m^{-2})	Plant fresh weight (g $plant^{-1}$)	Dry matter content (%)	Stomatal conductance (mmol m^{-2} s^{-1})	Leaf greenness (SPAD units)
Ebb-and-flow	2.3 ± 0.3 a	15.1 ± 1.9 a	9.6 ± 0.5 a	211.3 ± 5.6 a	23.1 ± 0.1 a
Aeroponic	2.2 ± 0.1 a	17.0 ± 0.6 a	9.3 ± 0.2 a	206.9 ± 8.8 a	23.1 ± 0.1 a

3.2. Plants growth and water use efficiency: comparison between ebb-and-flow and aeroponics

With regards to growth, plants cultivated in the two growing systems subjected to the same environmental conditions reported comparable growth. Indeed, yield values were between 2.3 and 2.2 kg m^{-2} and leaf fresh weight ranged 15.1–17.0 g $plant^{-1}$ (Table 5), in ebb-and-flow and aeroponics, respectively.

In previous research, yields of different growing systems presented different results. According to Chandra et al. (2014), aeroponic cultivation (developed in aeroponic towers located outdoor) of several crops, such as basil, parsley, cherry tomato, squash, bell pepper and kale increased from 19 % to 65 % as compared to on-soil open field cultivation. Comparing lettuce growth performances in a Venlo type glasshouse, Li et al. (2018) reported a greater shoot fresh weight for two lettuce cultivars ("Nenglv naiyou" and "Dasusheng") when cultivated in a nutrient film technique system, as compared to the aeroponic one. At the same time, both varieties showed an increase in the root:shoot ratio, when grown in the aeroponic system, meaning that more biomass was allocated to the roots. On the other hand, Puccinelli et al. (2021) showed a greater fresh weight in lettuces grown in aeroponics as compared to those grown in a floating system (with differences ranging 47–57 % depending on the experimental treatment). In the same experiment, no differences were found in basil plants grown in the two systems. These inconsistent trends can be however explained by the different fertigation regimes used in the trials. Indeed, the optimization of aeroponics depends on several variables, e.g., the droplet size, the pressure and the frequency for delivering the nutrient solution to the plants (Li et al., 2018), aspects that can influence plants' growth. This is particularly noteworthy considering that aeroponics is a less adopted technology and that optimal fertigation strategies for different crops are still poorly explored. Moreover, aeroponics is often claimed to make oxygen more accessible at roots level, as compared to other typology of substrate culture (Eldridge et al., 2020; Tunio et al., 2021). In our study, the use of an ebb-and-flow system may have avoided the problem of low oxygen availability that can be a limiting factor for growth in hydroponic cultures, especially in system such as the DWC. In our results, no statistically significant differences were found in dry matter content (mean value of 9.5 %) and in the parameters related to the photosynthetic performances, such as stomatal conductance (mean value of 209 mmol m^{-2} s^{-1}) and relative chlorophyll content associated with leaf greenness (23.1 SPAD units) (Table 5).

Concerning WUE results, the aeroponic system was found to be more efficient than the ebb-and-flow (Fig. 2). Considering only the water used for irrigation (W_i), aeroponics achieved a WU_iE of 69.7 g FW L^{-1} H_20 as compared to 32.5 g FW L^{-1} H_20 for the ebb-and-flow, thus leading to a 114 % increase (Fig. 2 A). Taking also into account the water used by the HVAC system and water for operations as inputs (WU_t), WU_tE drops by 14 % and 24 %, reaching values of 28.1 g FW L^{-1} H_20 for ebb-and-flow and 52.9 g FW L^{-1} H_20 for aeroponics, respectively (Fig. 2B). When water recovered by dehumidifier was removed from total water used (WU_f), WU_fE increased up to a value of 86.0 g FW L^{-1} H_2O in the ebb-and-flow system and 161.9 g FW L^{-1} H_2O in the aeroponic one (Fig. 2 C), resulting in an augment of 206 %. These values are even much higher than those reported in literature for WUE in vertical farms, with the highest values corresponding to 80 g FW L^{-1} H_2O for lettuce cultivation, although with some variability according to the crop considered (Orsini et al., 2020).

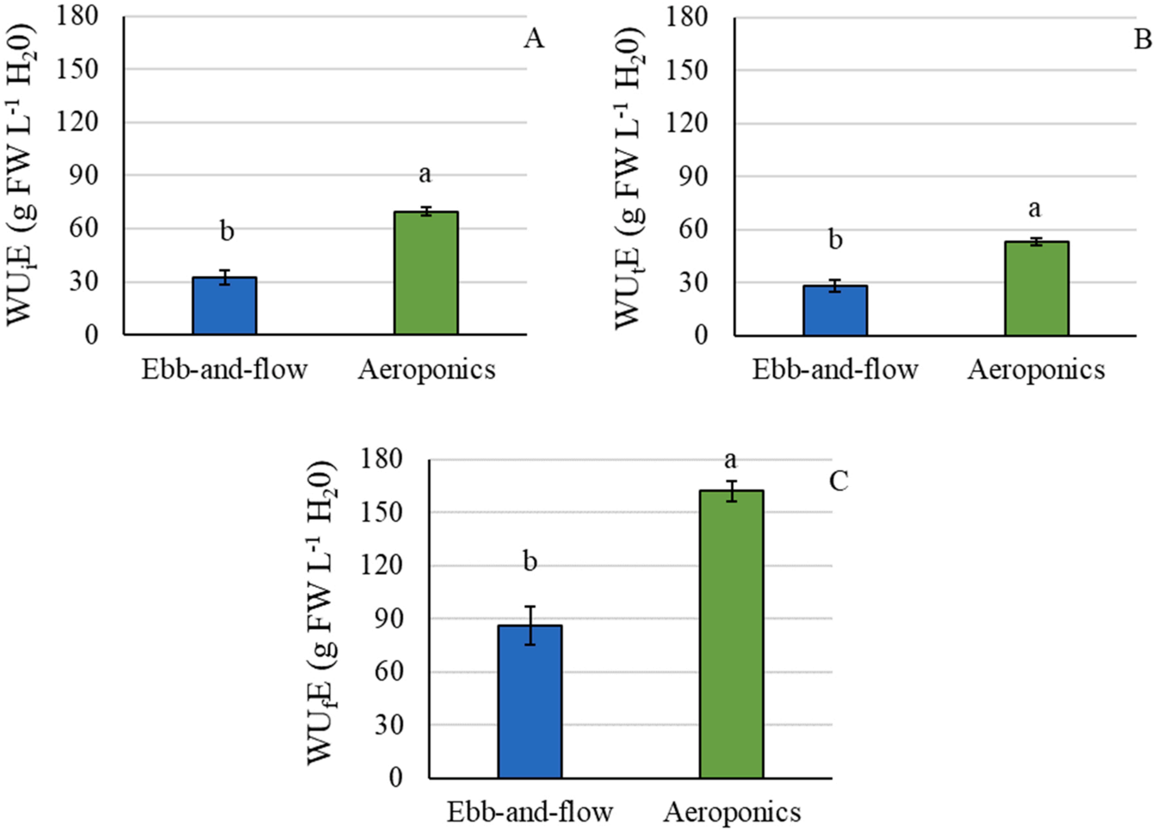

Fig. 2. Water Use Efficiency (g FW L^{-1} H_2O) obtained in the ebb-and-flow (blue) and aeroponic (green) systems under three different scenarios: A) considering as an input only water used for irrigation (WU_i); B) considering as an input water used for irrigation, climate control and management operations in the farm (WU_t); C) considering as an input water used for irrigation, climate control and management operations in the farm minus the water recovered from the dehumidifier (WU_f). Data ± standard error ($n = 15$) are reported. Different letters indicate significant differences according to the ANOVA test and the Tukey post-hoc test for mean separation with $p < 0.05$.

As reported by our data, no differences have been found in plants' photosynthetic parameters (Table 5). Therefore, the differences in WUE between the two systems could be attributed to two main factors: 1) a more efficient assimilation of water and nutrients in the aeroponics (Tunio et al., 2021), and 2) the lack of substrate in the aeroponic system, that would otherwise retain a percentage of the water given as input, thus preventing its re-use in the close-loop system. Indeed, as reported by Kozai and Niu (2016), plant tissues and substrates embedded 16 % of the total water used. In particular, peat, the substrate used for this work, has one of the highest water-holding capacities when compared to other most commonly used substrate for indoor farming (Du et al., 2022). Therefore, in a close-loop system, the substrate may play an important role in terms of WUE, aspect that must be balanced with final yield and products quality, that may also be influenced by the typology of substrate used as shown for instance in red basil microgreens grown with different substrates (Bulgari et al., 2021).

Although the adoption of the two growing systems did not result in differences in terms of yield (Table 5), the management of certain root zone parameters represents an opportunity to optimize yield, especially in aeroponics. In fact, the aeroponic system allows for the application of specific cultivation techniques such as the application of CO_2 at root level, which can significantly increase lettuce yield, as shown in He et al. (2013).

3.3. *Effects of cultivation techniques on water use efficiency*

3.3.1. *Optimal management of light spectrum*

Yield, DMC, leaf greenness, stomatal conductance and LAI varied according to the specific amount of far-red radiation in the spectrum. Enriching the spectrum with far-red radiation has demonstrated to increase leaf fresh weight, and therefore yield, of lettuce plants. Lee et al. (2016), for the cultivar "Sunmang", changing the red/far-red ratio from 0.7 to 4.1, reported an increase ranging 39–50 % as compared to red and blue spectrum, while Zou et al. (2019) (for cultivar "Tiberious") found a 56 % increase adding 50 μmol m^{-2} s^{-1} of far-red radiation to a red and blue spectrum. According to our results, yield already increased with RB_3-30 (+46 %) reaching the highest value with RB_3-70 (+64 %), as compared to RB_3 (Fig. 3).

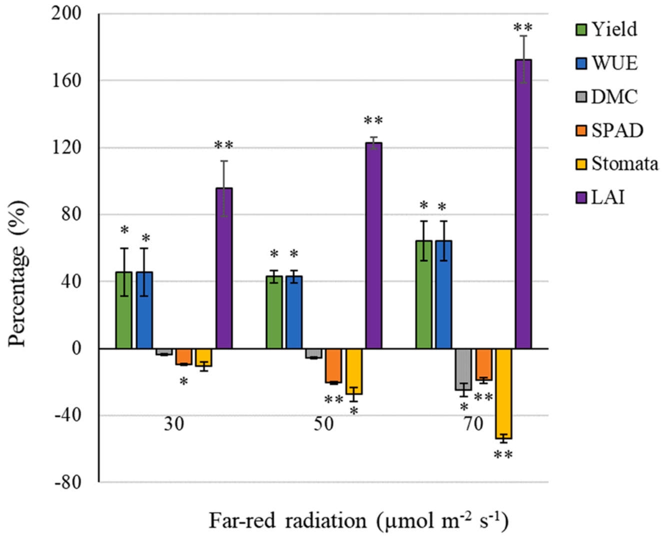

Fig. 3. Yield (kg FW m^{-2}), water use efficiency (WUE; kg FW L^{-1} H_20), dry matter content (DMC; %), leaf greenness (SPAD, SPAD units), stomatal conductance (Stomata; mmol m^{-2} s^{-1}) and leaf area index (LAI, m^2 m^{-2}) for plants grown with the three light treatments: RB_3-30, RB_3-50 and RB_3-70. WUE has been calculated by using WU_f according to formula 3 (details in **Section 2.5**). Values (data ± standard error, n = 15) are represented as percentage differences from the control, RB_3 treatment. The asterisks indicate the presence of significant differences as compared to RB_3, according to the ANOVA test, for $p < 0.05$ (*) or $p < 0.01$ (**). The control treatment values (RB_3) are the ones shown in Table 5 for the ebb-and-flow system.

The increase in leaf area is one of the most characterized plants responses in presence of far-red radiation, representing one of the main features of the shade avoidance response (Tan et al., 2022). In the hereby presented results, this effect was confirmed: LAI increased by 95 % by adding 30 μmol m^{-2} s^{-1} of far-red radiation up to 173 % with 70 μmol m^{-2} s^{-1} (Fig. 3). The presence of far-red radiation has been found to be beneficial, specifically at certain growth stages, such as in seedlings, for increasing biomass through the enhancement of leaf area, that can facilitate the light interception, as shown in Jin et al. (2021) and Park and Runkle (2017).

The effect of blue light in enhancing stomatal activity and thus reducing WUE has already been well documented in literature (Pennisi et al., 2019; Clavijo-Herrera et al., 2018). Our results suggest a potential influence of far-red radiation on stomatal conductance, with a significant reduction by 27 % and 54 % when RB_3-50 and RB_3-70 were used, as compared to the control. This result thus indicates that up to 30 μmol m^{-2} s^{-1} of far-red radiation replacing the same amount of red and blue radiation in the spectrum, do not affect the stomatal conductance (Fig. 3). Zhen and Bugbee (2020), adding the same amount of white or far-red photons to the spectrum, reported a lower stomatal conductance in the case of far-red radiation, suggesting that far-red decreases stomata opening as compared to white radiation. Same results were also found in tomato plants exposed to far-red radiation (Kalaitzoglou et al., 2019). Although different works reported a reduction in the stomatal conductance in presence of far-red radiation, the effect of far-red on this physiological activity has not yet been clarified (Tan et al., 2022). The trends described in this work could be the results of fewer blue photons in the applied spectrum, as a result of the substitution with far-red, or even a direct action of far-red on stomatal closure. Indeed, some studies performed on *Arabidopsis* speculate that far-red radiation may reverse the action of blue light on stomata activity (Talbott et al., 2003).

An interesting trend was observed for the leaf greenness (relative chlorophyll content), which decreased by 10–21 % increasing the percentage of far-red from 0 to 30–70 μmol m^{-2} s^{-1} (Fig. 3). A reduction in the chlorophyll content is often attributed to a "dilution effect" whereby leaf expansion is stimulated by far-red radiation at the expense of total organic nitrogen in the leaves (Mickens et al., 2018; Li and Kubota, 2009). Zou et al. (2021) reported this effect already with low far-red radiation in the spectrum (e.g., 15 μmol m^{-2} s^{-1}). The same trend was found in our results, where the effects of far-red radiation on relative chlorophyll content was already statistically significant upon 30 μmol m^{-2} s^{-1}, leading to a decrease (−10 %) (Fig. 3). The effect of far-red radiation on photosynthetic process is still a matter of debate since some authors have reported an increase in the photosynthetic activity in the presence of these wavelengths (Zhen and van Iersel, 2017), while as claimed by other authors the observed increase in yield is mainly a consequence of the change of certain morphological parameters (e.g., specific leaf area) (Jin et al., 2021).

According to our results, with the substitution of 30 and 50 μmol m^{-2} s^{-1} of far-red radiation in the spectrum, DMC remained unchanged (even if the yield was increased), while with 70 μmol m^{-2} s^{-1}, it reported a 25 % decrease (Fig. 3). Thus, a high amount of far-red in the spectrum could influence water accumulation processes within tissues, explaining the increase in yield that was still found with RB_3-70. Indeed, a reduction in photosynthetic performances in the presence of greater amount of far-red radiation (RB_3-70) can be hypothesized from the results, as confirmed by the observed reduced stomatal conductance and chlorophyll content as well as the lower dry matter content (Fig. 3). On the other hand, up to 50 μmol m^{-2} s^{-1} of far-red radiation in the spectrum replacing the same intensity of red and blue radiation, seemed not to reduce the photosynthetic rate when compared to the spectrum composed of only red and blue radiation (RB_3). In literature, an increase in dry matter towards the aerial plants' organs is reported at low R:FR ratio (Demotes-Mainard et al., 2016), with a greater allocation to the stem, as reported for different species by Poorter et al. (2012).

The results of this work highlighted that the precise management of far-red in the spectrum can also affect the overall WUE of the system, with an increase ranging 46–64 %, with the highest value achieved with the greatest amount of far-red in the spectrum (RB_3-70) (Fig. 3). To conclude, the results showed that: 1) different morphological and physiological responses may require a different "threshold" level of far-red radiation to be activated and 2) replacing 30–50 μmol m^{-2} s^{-1} of red and blue radiation with far-red radiation in the spectrum (15–25 % of the total radiation in the spectrum) can allow for considerable increases in WUE without compromising the DMC of the crops.

3.3.2. Optimal management of planting density

The use of three different planting densities resulted in different plants' growth at 20 and 33 DAT. At 20 DAT, the highest planting density (733 plants m^{-2}) led to an increase in yield (+143 %) as compared to the planting density used as a control (153 plants m^{-2}), but also at the lowest plant fresh weight (2.4 g $plant^{-1}$) (Table 6). At 33 DAT, the adoption of a planting density of 270 plants m^{-2} resulted in a 91 % increase in terms of yield as compared to the control (Table 6). Also in that case, the significantly lowest value of plant fresh weight was obtained at the highest planting density (7.3 ± 0.3 g $plant^{-1}$). On both harvest days, the dry matter content was not affected by the adopted planting density (Table 6). At 20 DAT, WUE at the highest planting density increased by 133 % as compared to the lowest one (Fig. 4), reaching 68.3 ± 14.3 g FW L^{-1} H_2O. At 33 DAT, the highest WUE was found at 270 and 733 plants m^{-2}, accounting for 135.0 ± 9.4 and 126 ± 10.0 g FW L^{-1} H_2O, respectively, and resulting in an increase of + 62–72 % as compared to the lowest planting density (Fig. 4).

The range of densities used in vertical farms is quite wide, with the average density being around 140 plants m^{-2} (Jin et al., 2022), similar to the one used as control in this study. The results of our study showed that using higher densities, especially in the early stages of growth, can lead to a significant increase in WUE (Fig. 4) without compromising dry matter content (Table 6).

Studies on lettuce growth model have reported that the optimal planting density during the growth cycle is the one for which leaf area index remains constant, thus increasing the distance between plants as they grown (Ioslovich and Gutman, 2000). While this strategy has been analyzed with regard to light use efficiency (Nicole et al., 2016), the results of this study showed that adapting the planting density through the growth cycle can lead to a considerable increase in WUE, making the practice of dynamic planting density also efficient in terms of use of resources other than land. Considering that the planting density in a vertical farm can be fully controlled, although the applicability of high densities and dynamic densities depends largely on the level of automation used, as suggested in van Delden et al. (2021), this cultivation technique is also effective in terms of resource use efficiency. Moreover, as reported by the simulation performed by Lalonde et al. (2019), the increase in cultivation density decreases the need of humidification by the system, since plants evapotranspiration affects the humidity level of the indoor environment. By increasing planting density there could be then the possibility to decrease the water input required for the humidification, since as it is shown from our data, 32 % of the total water is used by the HVAC system (Table 4). On the other hand, considering that the rate of crop-induced heat gain and loss is not negligible (Talbot and Monfet, 2020), increasing the planting density could also affect the cooling requirements of the HVAC system (Lalonde et al., 2019).

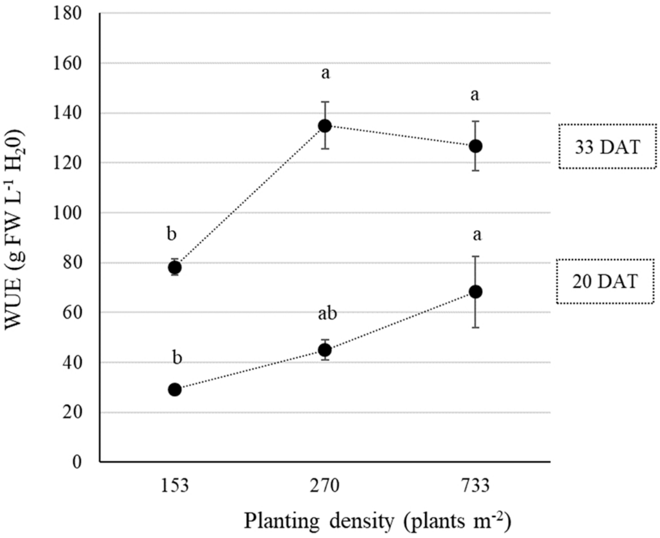

Fig. 4. Water Use Efficiency (WUE; g FW L^{-1} H_20 ± standard error) obtained by the use of the different planting density (153, 270 and 733 plants m^{-2}) at 20 and 33 DAT. WUE has been calculated by using WU_f according to formula 3. Data ± standard error (n = 15) are reported. Different letters indicate significant differences according to the ANOVA test and the Tukey post-hoc test for mean separation with $p < 0.05$.

Overall, what emerges from these scenarios, is that in terms of resource use efficiency, the next fundamental steps will require the study and characterization of cultivation techniques efficient in the trade-off between water and energy consumption. In some crops such as green lettuce, multiple harvests allow the crops to achieve higher fresh and dry weight (Carillo et al., 2021; Thapa et al., 2022), therefore with the aim to identifying the optimal density that takes into account various aspects of cultivation, it will be crucial to study a possible interaction between the use of high densities and multiple harvests, as well as the qualitative parameters of crops in case of high planting densities.

4. Conclusions

In a vertical farm with artificial light, the adoption of high pressure aeroponics and ebb-and-flow systems resulted in the same final lettuce yield. However, the use of the aeroponic system resulted in an increase

Table 6
Yield (kg FW m^{-2}), plant fresh weight (g $plant^{-1}$) and dry matter content (%) for plants grown with the three planting densities, at 20 and 33 days after transplanting (DAT): 153, 270 and 733 plants m^{-2}. Data ± standard errors (n = 15) are reported. Different letters indicate significant differences according to the ANOVA test and the Tukey post-hoc test for mean separation with $p < 0.05$.

DAT	Planting density (plants m^{-2})	Yield (kg m^{-2})	Plant fresh weight (g $plant^{-1}$)	Dry matter content (%)
20	153	0.7 ± 0.05 b	4.8 ± 0.2 a	5.5 ± 0.1 a
	270	1.3 ± 0.1 ab	4.7 ± 0.4 a	5.2 ± 0.1 a
	733	1.7 ± 0.4 a	2.4 ± 0.5 b	5.6 ± 0.2 a
33	153	3.3 ± 0.3 b	21.3 ± 0.1 a	6.2 ± 0.8 a
	270	6.3 ± 0.8 a	23.1 ± 2.1 a	5.9 ± 0.3 a
	733	5.4 ± 0.7 a	7.3 ± 0.3 b	6.0 ± 0.4 a

of water use efficiency (WUE) by 53 %. WUE values increased significantly (+206 %) also thanks to the recovery of water transpired by plants from the HVAC system, dramatically impacting the water requirement for irrigation. Applying specific cultivation techniques, such as substituting from 30 to 50 μmol m^{-2} s^{-1} (15–25 % of the total radiation) of red and blue radiation with the same amount of far-red radiation, increased WUE by 46–64 %, without affecting the crop dry matter content. Moreover, using different planting densities depending on the growth stage of the plants (≥ 270 plants m^{-2}), allowed to greatly increase WUE ranging 62–133 %. The results of this study therefore showed how the use of innovative technologies and specific cultivation techniques, e.g., the optimal management of far-red radiation and planting density, can contribute greatly to optimizing water use in an indoor growing system. In order to further optimize water use in a vertical farm, future studies should deeply characterize the consumption of the HVAC system when different cultivation strategies are adopted, taking into account the contribution in terms of both energy and water consumption, to improve the trade-off between different resources.

Funding

This work was supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 862663, by the Italian Ministry of Education and Research (MUR), within the call for Research Projects of National Interest (PRIN), within the project "VFARM - Sustainable Vertical Farming" (Project code: 2020ELWM82, CUP: J33C20002350001), and by the Swedish Research Council for Sustainable Development FORMAS (Grant Number: 2021-02447) and the Swedish Innovation Agency VINNOVA, (Grant Number: 2019-03178).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Francesco Orsini reports financial support was provided by European Union. Francesco Orsini reports financial support was provided by Government of Italy Ministry of Education University and Research. Michael Martin reports financial support was provided by Swedish Research Council Formas. Michael Martin reports financial support was provided by Sweden's Innovation Agency. Davide Meneghello reports a relationship with FRM srl that includes: employment.

Data Availability

Data will be made available on request.

References

Agovino, M., Casaccia, M., Ciommi, M., Ferrara, M., Marchesano, K., 2019. Agriculture, climate change and sustainability: the case of EU-28. Ecol. Indic. 105, 525–543. https://doi.org/10.1016/j.ecolind.2018.04.064.

Algarni, S., Saleel, C.A., Mujeebu, M.A., 2018. Air-conditioning condensate recovery and applications—current developments and challenges ahead. Sustain. Cities Soc. 37, 263–274. https://doi.org/10.1016/j.scs.2017.11.032.

Al-Kodmany, K., 2018. The vertical farm: a review of developments and implications for the vertical city. Buildings 8, 24. https://doi.org/10.3390/buildings8020024.

Appolloni, E., Pennisi, G., Zauli, I., Carotti, L., Paucek, I., Quaini, S., Orsini, F., Gianquinto, G., 2021. Beyond vegetables: effects of indoor LED light on specialized metabolite biosynthesis in medicinal and aromatic plants, edible flowers, and microgreens. J. Sci. Food Agric. 102, 472–487. https://doi.org/10.1002/jsfa.11513.

Avgoustaki, D.D., Xydis, G., 2021. Energy cost reduction by shifting electricity demand in indoor vertical farms with artificial lighting. Biosyst. Eng. 211, 219–229. https://doi.org/10.1016/j.biosystemseng.2021.09.006.

Bulgari, R., Negri, M., Santoro, P., Ferrante, A., 2021. Quality evaluation of indoor-grown microgreens cultivated on three different substrates. Horticulturae 7, 96. https://doi.org/10.3390/horticulturae7050096.

Carillo, P., Soteriou, G.A., Kyriacou, M.C., Giordano, M., Raimondi, G., Napolitano, F., Di Stasio, E., Mola, I.D., Mori, M., Rouphael, Y., 2021. Regulated salinity eustress in a floating hydroponic module of sequentially harvested lettuce modulates phytochemical constitution, plant resilience, and post-harvest nutraceutical quality. Agronomy 11, 1040. https://doi.org/10.3390/agronomy11061040.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M.H., ElSohly, M.A., Khan, I.A., 2014. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. Evid. Based Complement. Altern. Med. 2014, 1–9. https://doi.org/10.1155/2014/253875.

Clavijo-Herrera, J., van Santen, E., Gómez, C., 2018. Growth, water-use efficiency, stomatal conductance, and nitrogen uptake of two lettuce cultivars grown under different percentages of blue and red light. Horticulturae 4, 16. https://doi.org/10.3390/horticulturae4030016.

van Delden, S.H., SharathKumar, M., Butturini, M., Graamans, L.J.A., Heuvelink, E., Kacira, M., Kaiser, E., Klamer, R.S., Klerkx, L., Kootstra, G., Loeber, A., Schouten, R. E., Stanghellini, C., van Ieperen, W., Verdonk, J.C., Vialet-Chabrand, S., Woltering, E.J., van de Zedde, R., Zhang, Y., Marcelis, L.F.M., 2021. Current status and future challenges in implementing and upscaling vertical farming systems. Nat. Food 2, 944–956. https://doi.org/10.1038/s43016-021-00402-w.

Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., Crespel, L., Morel, P., Huché-Thélier, L., Boumaza, R., Vian, A., Guérin, V., Leduc, N., Sakr, S., 2016. Plant responses to red and far-red lights, applications in horticulture. Environ. Exp. Bot. 121, 4–21. https://doi.org/10.1016/j.envexpbot.2015.05.010.

Du, M., Xiao, Z., Luo, Y., 2022. Advances and emerging trends in cultivation substrates for growing sprouts and microgreens toward safe and sustainable agriculture. Curr. Opin. Food Sci. 46, 100863 https://doi.org/10.1016/j.cofs.2022.100863.

Eldridge, B.M., Manzoni, L.R., Graham, C.A., Rodgers, B., Farmer, J.R., Dodd, A.N., 2020. Getting to the roots of aeroponic indoor farming. N. Phytol. 228, 1183–1192. https://doi.org/10.1111/nph.16780.

FAO, 2014. The water-energy-food nexus. A New Approach in Support of Food Security and Sustainable Agriculture. Food and Agricultural Organization of the United Nations, Rome, Italy. ⟨https://www.fao.org/3/bl496e/bl496e.pdf⟩.

Graamans, L., Baeza, E., van den Dobbelsteen, A., Tsafaras, I., Stanghellini, C., 2018. Plant factories versus greenhouses: comparison of resource use efficiency. Agric. Syst. 160, 31–43. https://doi.org/10.1016/j.agsy.2017.11.003.

Gruda, N.S., 2020. Soilless culture systems and growing media in horticulture: an overview. Advances in horticultural soilless culture. Burleigh Dodds Science Publishing. https://doi.org/10.1201/9781003048206.

He, J., Qin, L., Lee, S.K., 2013. Root-zone CO_2 and root-zone temperature effects on photosynthesis and nitrogen metabolism of aeroponically grown lettuce (*Lactuca sativa* L.) in the tropics. Photosynt 51, 330–340. https://doi.org/10.1007/s11099-013-0030-5.

Incrocci, L., Thompson, R.B., Fernandez-Fernandez, M.D., De Pascale, S., Pardossi, A., Stanghellini, C., Rouphael, Y., Gallardo, M., 2020. Irrigation management of European greenhouse vegetable crops. Agric. Water Manag. 242, 106393 https://doi.org/10.1016/j.agwat.2020.106393.

Ioslovich, I., Gutman, P.O., 2000. Optimal control of crop spacing in a plant factory. Automatica 36, 1665–1668.

Jin, W., Urbina, J.L., Heuvelink, E., Marcelis, L.F.M., 2021. Adding far-red to red-blue light-emitting diode light promotes yield of lettuce at different planting densities. Front. Plant Sci. 11, 609977 https://doi.org/10.3389/fpls.2020.609977.

Jin, W., Formiga Lopez, D., Heuvelink, E., Marcelis, L.F.M., 2022. Light use efficiency of lettuce cultivation in vertical farms compared with greenhouse and field. Food Energy Secur. 12, e39 https://doi.org/10.1002/fes3.391.

Jurga, A., Pacak, A., Pandelidis, D., Kaźmierczak, B., 2023. Condensate as a water source in terrestrial and extra-terrestrial conditions. Water Resour. Ind. 29, 100196 https://doi.org/10.1016/j.wri.2022.100196.

Kalaitzoglou, P., van Ieperen, W., Harbinson, J., van der Meer, M., Martinakos, S., Weerheim, K., Nicole, C.C.S., Marcelis, L.F.M., 2019. Effects of continuous or end-of-day far-red light on tomato plant growth, morphology, light absorption, and fruit production. Front. Plant Sci. 10, 322. https://doi.org/10.3389/fpls.2019.00322.

Kalantari, F., Tahir, O.M., Joni, R.A., Fatemi, E., 2018. Opportunities and challenges in sustainability of vertical farming: a review. J. Landsc. Ecol. 11, 35–60. https://doi.org/10.1515/jlecol-2017-0016.

Katsoulas, N., Sapounas, A., De Zwart, F., Dieleman, J.A., Stanghellini, C., 2015. Reducing ventilation requirements in semi-closed greenhouses increases water use efficiency. Agric. Water Manag. 156, 90–99. https://doi.org/10.1016/j.agwat.2015.04.003.

Kozai, T., Niu, G., 2016. Plant factory as a resource-efficient closed plant production system. Plant factory. Elsevier,, pp. 69–90. https://doi.org/10.1016/B978-0-12-801775-3.00004-4.

Kusuma, P., Bugbee, B., 2021. Far-red Fraction: an improved metric for characterizing phytochrome effects on morphology. J. Am. Soc. Hort. Sci. 146, 3–13. https://doi.org/10.21273/JASHS05002-20.

Lakhiar, I.A., Gao, J., Syed, T.N., Chandio, F.A., Buttar, N.A., 2018. Modern plant cultivation technologies in agriculture under controlled environment: a review on aeroponics. J. Plant Interact. 13, 338–352. https://doi.org/10.1080/17429145.2018.1472308.

Lalonde, T., Talbot, M.-H., Monfet, D., 2019. The Impact of Plants on HVAC System Performance in Cold Climate: a Parametric Study. Presented at the Building Simulation 2019, Rome, Italy, pp. 1921–1928. https://doi.org/10.26868/25222708.2019.210721.

Langenfeld, N.J., Pinto, D.F., Faust, J.E., Heins, R., Bugbee, B., 2022. Principles of nutrient and water management for indoor agriculture. Sustainability 14, 10204. https://doi.org/10.3390/su141610204.

Lee, M.-J., Son, K.-H., Oh, M.-M., 2016. Increase in biomass and bioactive compounds in lettuce under various ratios of red to far-red LED light supplemented with blue LED light. Hortic. Environ. Biotechnol. 57, 139–147. https://doi.org/10.1007/s13580-016-0133-6.

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011.

Li, Q., Li, X., Tang, B., Gu, M., 2018. Growth responses and root characteristics of lettuce grown in aeroponics, hydroponics, and substrate culture. Horticulturae 4, 35. https://doi.org/10.3390/horticulturae4040035.

Lovichit, W., Kubota, C., Choi, C.Y., Schoonderbeek, J., 2007. Greenhouse water recovery system for crop production in semi-arid climate. ASABE Paper No. 074012. Presented at the 2007 ASAE Annual Meeting. ASABE, St. Joseph, Michigan. https://doi.org/10.13031/2013.23053.

Malhi, G.S., Kaur, M., Kaushik, P., 2021. Impact of climate change on agriculture and its mitigation strategies: a review. Sustainability 13, 1318. https://doi.org/10.3390/su13031318.

Massa, G.D., Kim, H.-H., Wheeler, R.M., Mitchell, C.A., 2008. Plant productivity in response to led lighting. HortScience 43, 1951–1956. https://doi.org/10.21273/HORTSCI.43.7.1951.

McCree, K.J., 1971. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9, 191–216. https://doi.org/10.1016/0002-1571(71)90022-7.

Michelon, N., Pennisi, G., Myint, N., Orsini, F., Gianquinto, G., 2021. Optimization of substrate and nutrient solution strength for lettuce and chinese cabbage seedling production in the semi-arid environment of central myanmar. Horticulturae 7, 64. https://doi.org/10.3390/horticulturae7040064.

Mickens, M.A., Skoog, E.J., Reese, L.E., Barnwell, P.L., Spencer, L.E., Massa, G.D., Wheeler, R.M., 2018. A strategic approach for investigating light recipes for 'Outredgeous' red romaine lettuce using white and monochromatic LEDs. Life Sci. Space Res. 19, 53–62. https://doi.org/10.1016/j.lssr.2018.09.003.

Nicole, C.C.S., Charalambous, F., Martinakos, S., van de Voort, S., Li, Z., Verhoog, M., Krijn, M., 2016. Lettuce growth and quality optimization in a plant factory. Acta Hortic. 231–238. https://doi.org/10.17660/ActaHortic.2016.1134.31.

Ohyama, K., Kozai, T., Yoshinaga, K., 2000. Electric energy, water and carbon dioxide utilization efficiencies of closed-type transplant production system. Transplant production in the 21st Century. Kluwer Academic Publishers, pp. 28–32. https://doi.org/10.1007/978-94-015-9371-7_3.

Ohyama, K., Yamaguchi, J., Enjoji, A., 2020. Resource utilization efficiencies in a closed system with artificial lighting during continuous lettuce production. Agronomy 10, 723. https://doi.org/10.3390/agronomy10050723.

Orsini, F., Pennisi, G., Zulfiqar, F., Gianquinto, G., 2020. Sustainable use of resources in plant factories with artificial lighting (PFALs). Eur. J. Hortic. Sci. 85, 297–309. https://doi.org/10.17660/eJHS.2020/85.5.1.

Orsini, F., Carotti, L., Souri, M.K., Pennisi, G., Gianquinto, G., 2023. Optimizing energy and other resource use in vertical farm. In press. Advances in Plant Factories: New Technologies in Indoor Vertical Farming. Burleigh Dodds Series.

Pacak, A., Jurga, A., Drąg, P., Pandelidis, D., Kaźmierczak, B., 2020. A long-term analysis of the possibility of water recovery for hydroponic lettuce irrigation in indoor vertical farm. Part 1: Water recovery from exhaust air. Appl. Sci. 10, 8907. https://doi.org/10.3390/app1024890.7.

Park, Y., Runkle, E.S., 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Exp. Bot. 136, 41–49. https://doi.org/10.1016/j.envexpbot.2016.12.013.

Pennisi, G., Orsini, F., Blasioli, S., Cellini, A., Crepaldi, A., Braschi, I., Spinelli, F., Nicola, S., Fernandez, J.A., Stanghellini, C., Gianquinto, G., Marcelis, L.F.M., 2019. Resource use efficiency of indoor lettuce (*Lactuca sativa* L.) cultivation as affected by red:blue ratio provided by LED lighting. Sci. Rep. 9, 14127. https://doi.org/10.1038/s41598-019-50783-z.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. N. Phytol. 193, 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x.

Puccinelli, M., Landi, M., Maggini, R., Pardossi, A., Incrocci, L., 2021. Iodine biofortification of sweet basil and lettuce grown in two hydroponic systems. Sci. Hortic. 276, 109783 https://doi.org/10.1016/j.scienta.2020.109783.

Putra, P.A., Yuliando, H., 2015. Soilless culture system to support water use efficiency and product quality: a review. Agric. Agric. Sci. Proc. 3, 283–288. https://doi.org/10.1016/j.aaspro.2015.01.054.

Ramin Shamshiri, R., Kalantari, F., C. Ting, K., R. Thorp, K., A. Hameed, I., Weltzien, C., Ahmad, D., Mojgan Shad, Z., 2018. Advances in greenhouse automation and controlled environment agriculture: A transition to plant factories and urban agriculture. Int. J. Agric. Biol. Eng. 11, 1–22. https://doi.org/10.25165/j.ijabe.20181101.3210.

Rufí-Salís, M., Petit-Boix, A., Villalba, G., Sanjuan-Delmás, D., Parada, F., Ercilla-Montserrat, M., Arcas-Pilz, V., Muñoz-Liesa, J., Rieradevall, J., Gabarrell, X., 2020. Recirculating water and nutrients in urban agriculture: an opportunity towards environmental sustainability and water use efficiency. J. Clean. Prod. 261, 121213 https://doi.org/10.1016/j.jclepro.2020.121213.

Son, K.-H., Oh, M.-M., 2015. Growth, photosynthetic and antioxidant parameters of two lettuce cultivars as affected by red, green, and blue light-emitting diodes. Hortic. Environ. Biotechnol. 56, 639–653. https://doi.org/10.1007/s13580-015-1064-3.

Soussi, M., Chaibi, M.T., Buchholz, M., Saghrouni, Z., 2022. Comprehensive review on climate control and cooling systems in greenhouses under hot and arid conditions. Agronomy 12, 626. https://doi.org/10.3390/agronomy12030626.

Talbot, M.-H., Monfet, D., 2020. Estimating the impact of crops on peak loads of a Building-Integrated Agriculture space. Sci. Technol. Built Environ. 26, 1448–1460. https://doi.org/10.1080/23744731.2020.1806594.

Talbott, L.D., Shmayevich, I.J., Chung, Y., Hammad, J.W., Zeiger, E., 2003. Blue light and phytochrome-mediated stomatal opening in the *npq1* and *phot1 phot2* mutants of *Arabidopsis*. Plant Physiol. 133, 1522–1529. https://doi.org/10.1104/pp.103.029587.

Tan, T., Li, S., Fan, Y., Wang, Z., Ali Raza, M., Shafiq, I., Wang, B., Wu, X., Yong, T., Wang, X., Wu, Y., Yang, F., Yang, W., 2022. Far-red light: a regulator of plant morphology and photosynthetic capacity. Crop J. 10, 300–309. https://doi.org/10.1016/j.cj.2021.06.007.

Tavan, M., Wee, B., Brodie, G., Fuentes, S., Pang, A., Gupta, D., 2021. Optimizing sensor-based irrigation management in a soilless vertical farm for growing microgreens. Front. Sustain. Food Syst. 4, 622720 https://doi.org/10.3389/fsufs.2020.622720.

Thapa, U., Nandi, S., Rai, R., Upadhyay, A., 2022. Effect of nitrogen levels and harvest timing on growth, yield and quality of lettuce under floating hydroponic system. J. Plant Nutr. 45, 2563–2577. https://doi.org/10.1080/01904167.2022.2064299.

Tunio, M.H., Gao, J., Lakhiar, I.A., Solangi, K.A., Qureshi, W.A., Shaikh, S.A., Chen, J., 2021. Influence of atomization nozzles and spraying intervals on growth, biomass yield, and nutrient uptake of butter-head lettuce under aeroponics system. Agronomy 11, 97. https://doi.org/10.3390/agronomy11010097.

Wiebe, K., Robinson, S., Cattaneo, A., 2019. Climate change, agriculture and food security. Sustainable Food and Agriculture. Elsevier,, pp. 55–74. https://doi.org/10.1016/B978-0-12-812134-4.00004-2.

Yildiz, I., Stombaugh, D.P., 2006. Heat pump cooling and greenhouse microclimates in open and confined greenhouse systems. Acta Hortic. 255–262. https://doi.org/10.17660/ActaHortic.2006.719.28.

Zhen, S., Bugbee, B., 2020. Far-red photons have equivalent efficiency to traditional photosynthetic photons: Implications for redefining photosynthetically active radiation. Plant Cell Environ. 43, 1259–1272. https://doi.org/10.1111/pce.13730.

Zhen, S., van Iersel, M.W., 2017. Far-red light is needed for efficient photochemistry and photosynthesis. J. Plant Physiol. 209, 115–122. https://doi.org/10.1016/j.jplph.2016.12.004.

Zou, J., Zhang, Yating, Zhang, Yuqi, Bian, Z., Fanourakis, D., Yang, Q., Li, T., 2019. Morphological and physiological properties of indoor cultivated lettuce in response to additional far-red light. Sci. Hortic. 257, 108725 https://doi.org/10.1016/j.scienta.2019.108725.

Zou, J., Fanourakis, D., Tsaniklidis, G., Cheng, R., Yang, Q., Li, T., 2021. Lettuce growth, morphology and critical leaf trait responses to far-red light during cultivation are low fluence and obey the reciprocity law. Sci. Hortic. 289, 110455 https://doi.org/10.1016/j.scienta.2021.110455.