Version of record at: https://doi.org/10.1016/j.jafr.2025.102530

Journal of Agriculture and Food Research 24 (2025) 102530

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Optimizing germination and growth of greenhouse hydroponic baby spinach via substrate moisture, active aeration, and nitrogen concentration

Qingwu Meng [a,*], Kishan Biradar [a,1], Yujin Park [b], Patrick N. Kelley [a,1]

[a] Department of Plant and Soil Sciences, University of Delaware, 531 South College Avenue, Newark, DE, 19716, USA
[b] School of Applied Sciences and Arts, College of Integrative Sciences and Arts, Arizona State University, Mesa, AZ, 85212, USA

ARTICLE INFO

Keywords:
Controlled environment agriculture
Dissolved oxygen
Leafy greens
Soilless culture
Spinach cultivars

ABSTRACT

Uniform germination is essential for hydroponic baby spinach (*Spinacia oleracea*) production, yet poor and inconsistent seed germination remains a common problem. This study examined how the initial substrate moisture affected seed germination and how nutrient solution active aeration and nitrogen concentration influenced subsequent growth. Seeds of four cultivars ('Corvair', 'Kolibri', 'Seaside', and 'Space') were sown in foam trays filled with a peat-perlite substrate at four initial moisture indices [$weight_{water} \div (weight_{water} + weight_{substrate})$] of 0.33, 0.50, 0.67, and 0.75. Trays were incubated in darkness at ≈95.7 % relative humidity for 5 days, then transferred to greenhouse deep-water culture for 15 days at ≈21.2 °C air temperature and ≈40.7 % relative humidity under sunlight with supplemental lighting (daily light integral ≈17.3 mol m^{-2} d^{-1}, 16-h photoperiod). During this phase, plants were grown in a split-split-plot randomized complete block design, with 12 flood tables divided into two blocks, receiving six nutrient solution treatments (125, 175, or 225 mg L^{-1} nitrogen × with or without active aeration). Germination improved with decreasing substrate moisture index; at 0.33, all cultivars showed higher germination than at 0.67 or 0.75. 'Kolibri' had the highest germination (87 %) at 0.33 by day 8. Trays with higher germination also produced greater total shoot fresh mass. Active aeration increased individual shoot fresh mass by 59 %–143 % and enhanced total yield, while nitrogen concentration above 125 mg L^{-1} provided little or negative benefit. Overall, a low initial substrate moisture index (0.33) enhanced germination, and post-germination growth was optimized by active aeration and moderate nitrogen supply.

1. Introduction

Most controlled-environment agriculture operations center around soilless production of fast-growing and high-value leafy greens, culinary herbs, and fruits, including lettuce (*Lactuca sativa*), basil (*Ocimum basilicum*), and tomato (*Solanum lycopersicum*). Known for its high nutritional value and health benefits, spinach (*Spinacia oleracea*) is becoming an increasingly popular packaged salad green consumed as baby leaves [1]. Despite high market demand, spinach was not established as a common greenhouse hydroponic crop until recently, primarily due to its high susceptibility to the waterborne oomycete *Pythium aphanidermatum* [2]. This pathogen spreads easily from root to root in hydroponic nutrient solutions, causing stunted growth and root rot [2]. With proper disease management protocols, some commercial greenhouses and indoor vertical farms have been successful in cultivating hydroponic baby spinach as a commercial product [3]. In greenhouses, baby spinach is typically grown at a high planting density in substrate-filled expanded polystyrene trays floating in a nutrient solution pond (deep-water culture) for a few weeks from seed to harvest [4].

Fast, consistent, and uniform germination is critical to hydroponic baby spinach production; however, spinach seeds do not germinate well without special attention paid to their unique seed structure and the germination environment. The true seed of spinach has the pericarp, which is a hard-shell enclosure with partially lignified cells [5]. The pericarp is a physical barrier for radicle emergence and limits oxygen availability to the true seed, which is vital to germination by supporting embryo respiration [6]. Mechanical, liquid, or chemical treatments that remove, crack, or soften the pericarp can improve spinach seed germination [6,7]. Spinach seeds are also sensitive to both high and low moisture content resulting in inconsistent germination [8]. For

* Corresponding author. Department of Plant and Soil Sciences, University of Delaware, 531 South College Avenue, Newark, DE 19716, USA.
E-mail address: qwmeng@udel.edu (Q. Meng).
[1] K.B. is a former graduate student at the University of Delaware. P.N.K. is a former lab volunteer, although not employed by the University of Delaware.

https://doi.org/10.1016/j.jafr.2025.102530
Received 12 June 2025; Received in revised form 12 November 2025; Accepted 15 November 2025
Available online 17 November 2025

peat-based substrates, two protocols for spinach seed germination recommended substrate-to-water ratios of 1:3 (w/w) [4] and 1:0.1 (w/w, estimated) [9]. However, our preliminary test using a peat-perlite substrate showed that each ratio resulted in either water-saturated or water-deficient conditions, respectively, indicating that the moisture content needs to be adjusted to suit specific factors, such as the substrate type.

Adequate dissolved oxygen in the root zone ensures optimal metabolic processes and root respiration, which provides energy for active nutrient uptake in plants [10]. The dissolved oxygen level of a standing hydroponic system (e.g., deep-water culture) full of plants can decrease rapidly if external oxygen is not supplemented in the nutrient solution [11]. Additional dissolved oxygen is typically delivered with active aeration, which uses an external air pump to disperse ambient air or pure oxygen gas through air stones into the nutrient solution. This is of particular importance during warm or hot seasons because dissolved oxygen decreases with increasing temperatures [12]. The extent to which the dissolved oxygen level influences hydroponic plant growth varies by species. In deep-water culture, varying the dissolved oxygen level did not affect the growth of lettuce 'Ostinata', whereas elevated dissolved oxygen increased the growth of spinach 'Paruku', 'S. Marco', 'S. Felix', and 'Dolphin RZ' and pepper (*Capsicum annuum*) 'California Wonder' ([11,13,14], Tesi 2002).

As an essential macronutrient from fertilizers, nitrogen should be supplied to plants sufficiently, but not excessively, to maximize nitrogen use efficiency, conserve fertilizer use, and prevent nitrate accumulation for human health [15,16]. Limited nitrogen availability restricts plant growth as nitrogen is an indispensable primary element in amino acids and proteins. The recommended nutrient solution nitrogen concentration for hydroponic leafy greens is generally 125 mg L^{-1} during propagation and 150 mg L^{-1} for light-to-moderate feeders (e.g., lettuce) or 175–200 mg L^{-1} for heavy feeders (e.g., spinach) during production [17,18]. In contrast, a nutrient solution nitrogen concentration of 112 mg L^{-1} was deemed optimal for hydroponic baby spinach either based on visual assessment or a limited test range [15,19]. Recent research on hydroponic baby spinach that does not study nitrogen has used inconsistent nitrogen concentrations ranging from 90 to 227 mg L^{-1} [20–22]. The optimal nutrient solution nitrogen concentration for different hydroponic baby spinach cultivars and whether it depends on active aeration remain unclear.

The objectives of this study were to determine 1) the optimal initial substrate-to-water ratio for spinach seed germination in a peat-perlite substrate; and 2) the effects of active aeration and nitrogen concentration of the hydroponic nutrient solution on the growth of four baby spinach cultivars in greenhouse deep-water culture. We postulated that 1) regardless of cultivars, the germination rate of spinach would be higher at moderately higher initial substrate-to-water ratios (w/w); and 2) increasing nitrogen concentration from 125 to 225 mg L^{-1} and/or providing active aeration in the hydroponic nutrient solution would increase baby spinach growth in greenhouse deep-water culture.

2. Materials and methods

2.1. Plant material and seed germination treatments

We obtained seeds of four spinach cultivars ('Corvair', 'Kolibri', 'Seaside', and 'Space') from a seed producer (Johnny's Selected Seeds, Winslow, ME, USA). We compared their germination rates in a peat-perlite growing substrate (PRO-MIX; Premier Tech Home and Garden, Quakertown, PA, USA) at four initial substrate-to-water ratios (1:0.5, 1:1, 1:2, and 1:3, w/w). Based on each of these ratios, a moisture index was calculated as $weight_{water} \div (weight_{water} + weight_{substrate}$, which ranges from 0 (when no added moisture is present in the substrate) to 1 (when there is only moisture, without any substrate). Therefore, seeds were subject to four moisture indices of 0.33, 0.50, 0.67, and 0.75, respectively.

On day 0, we prepared the substrate with the four initial substrate-to-water ratios by thoroughly mixing appropriate weights of the substrate and water. We then filled the substrate with the four initial substrate-to-water ratios into four sets of 12 foam trays (model TR338DT; Speedling, Inc., Sun City, FL, USA). Each foam tray originally had 338 square cells, (individual cell length = 2.1 cm, depth = 5.7 cm, volume = 14.7 cm^3); we trimmed two sides so that each foam tray had 253 cells in a 23 × 11 layout. This allowed four foam trays to be subsequently placed in each of 12 deep-water-culture flood tables [1.22 × 0.61 × 0.18 m (Active Aqua AAHR24W; Hydrofarm, Shoemakersville, PA, USA)] in a 2 × 2 layout. We divided each foam tray into four equal quadrants corresponding to the four cultivars which were separated by one empty row. The quadrant for each cultivar contains 55 cells in a 5 × 11 layout with one seed sown per cell.

Immediately following seed sowing, we stacked and covered the 48 foam trays (with four initial substrate-to-moisture ratios; 12 trays for each ratio) with opaque polyethylene sheets (Black and White Panda Film; Vivosun, Ontario, CA, USA) in a dark growth chamber at 24 °C to maintain a high relative humidity of 95.7 % ± 4.3 % (mean ± *SD*). Temperature and relative humidity were measured with a sensor (HOBO MX1101 Temp/RH logger; Onset Computer Corporation, Bourne, MA, USA). This condition lasted from day 0–5 until seedling emergence. On day 5, we recorded germination rates and continued to float and grow baby spinach plants in foam trays using greenhouse hydroponic deep-water culture.

2.2. Greenhouse hydroponic nutrient solution treatments

Upon seed germination on day 5, we removed the polyethylene sheets and relocated all foam trays to a glass-glazed, climate-controlled greenhouse to conduct the subsequent hydroponic nutrient solution experiment from day 5–20. Four foam trays with the four initial substrate-to-moisture ratios (with each foam tray containing the four spinach cultivars) floated in each of the 12 deep-water-culture flood tables, which were placed on concrete benches and contained 8-cm-deep nutrient solutions. In a split-split-plot randomized complete block design, the 12 flood tables were divided into two blocks (i.e., two replications from two benches) of six nutrient solution treatments, including three nutrient solution nitrogen concentrations (125, 175, and 225 mg L^{-1}), each without or with active aeration. All factors studied are summarized in Table 1.

The initial nutrient solution in each flood table consisted of 59.4 L of municipal water supplemented with a base fertilizer (5–12–26 Part A, JR Peters, Inc., Allentown, PA, USA) and calcium nitrate (15–0–0 Cal Nit Part B, JR Peters, Inc.). The amount of the base fertilizer was fixed at 1000 mg L^{-1} in all containers, whereas the amount of calcium nitrate was adjusted to 500, 833, and 1166 mg L^{-1} to provide the three nitrogen concentrations of 125, 175, and 225 mg L^{-1}, respectively. By increasing the nitrogen concentration with calcium nitrate, the respective calcium concentrations were 90, 150, and 210 mg L^{-1}, and total nutrient strength also increased correspondingly. Therefore, we cannot rule out the possibility that any effect interpreted as caused by increasing the

Table 1
A summary of all factors studied in a greenhouse hydroponic baby spinach experiment following a split-split-plot randomized complete block design. The moisture index is calculated as $weight_{water} \div (weight_{water} + weight_{substrate})$.

Spinach cultivar	Initial moisture treatment		Nutrient solution treatment	
	Substrate-to-water ratio (w/w)	Moisture index	Nitrogen concentration (mg L^{-1})	Active aeration
'Corvair'	1:0.5	0.33	125	No
'Kolibri'	1:1	0.50	175	Yes
'Seaside'	1:2	0.67	225	
'Space'	1:3	0.75		

nitrogen concentration could also be attributed to increasing the calcium concentration or total nutrient strength. Otherwise, all nutrient solutions had the same nutrient composition (in mg·L^{-1}): 52 phosphorus, 216 potassium, 63 magnesium, 85 sulfur, 0.50 boron, 0.15 copper, 3.00 iron, 0.50 manganese, 0.19 molybdenum, and 0.15 zinc. No water or fertilizers were added to any of the nutrient solutions during the experiment; therefore, the initial nutrient concentrations were not maintained, and depletion due to plant uptake was not controlled. This approach was intentional, allowing assessment of nutrient effects under unadjusted conditions. The nutrient solution volume was sufficiently large relative to the short growth period and small plant size to ensure an adequate nutrient supply throughout cultivation. We measured the nutrient solution pH in each flood table daily with a portable meter (HI9814; Hanna Instruments) and adjust the pH as needed to 5.8 ± 0.3, whenever outside this range, with diluted solutions of food-grade H_3PO_4 (pH Down; General Hydroponics, Inc., Santa Rosa, CA, USA), and KOH and K_2CO_3 (pH Up; General Hydroponics, Inc.).

Active aeration of the nutrient solution (with ambient air) in each of the six aerated flood tables was achieved by continuously (24 h d^{-1} during the entire greenhouse experiment) operating two evenly distributed, submerged air stone discs (12.7-cm diameter; Vivosun) connected via vinyl air tubing (Hydro Flow model 708220; Sunlight Supply Inc., Vancouver, WA, USA) to an external air pump (model 102W-1750GPH; Vivosun). Air valves were opened to the extent of reaching 100 % dissolved oxygen as measured in the center of each flood table by a portable meter (HI9147-04; Hanna Instruments, Smithfield, RI, USA).

In the middle of each greenhouse bench, we placed a quantum sensor (SQ-500; Apogee Instruments, Inc., Logan, UT, USA) to log the photosynthetic photon flux density every 10 min (based on the average of 1-min measurements) and a temperature and relative humidity sensor (HOBO MX2301; Onset Computer Corporation) to log data every 10 min. Throughout the greenhouse experiment, the photoperiod was 16 h, which was kept constant by using supplemental lighting from high-pressure sodium lamps in addition to natural sunlight. The mean daily light integral was 17.3 mol m^{-2} d^{-1}, measured at the top of the plant canopy. The air temperature and relative humidity (mean ± *SD*) were 21.2 ± 1.9 °C and 40.7 % ± 14.6 %, respectively.

2.3. Data collection and analysis

We counted the number of germinated seeds from each tray quadrant on day 8 and calculated the spinach germination rate by dividing the number of germinated seeds by the total number of seeds sown. The criterion for seed germination was the appearance of a visible radicle with a length greater than 0.5 cm. On day 20 (15 d after floating), we harvested baby spinach plants from each tray quadrant when leaves reached the marketable size (5–8 cm long). We measured individual shoot fresh mass for three representative plants in each tray quadrant, as well as total shoot fresh mass of all plants in each tray quadrant (for each cultivar) using a top-loading balance (Apollo GX-1003A; A&D Weighing, Ann Arbor, MI, USA). Morphological traits, including the length and width of the largest leaf per plant, were measured with a ruler. Leaf chlorophyll concentration index was determined for the largest leaf of three representative plants in each tray quadrant using a chlorophyll meter (MC-100, Apogee Instruments, Inc.). Data of each parameter per cultivar were pooled from both replications given similar treatment responses and overall lack of treatment × block interactions. We performed mean separations via pairwise comparisons in Tukey's honest significant difference test ($P \leq 0.05$) using statistical software JMP Pro (version 17.0.0; SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Visible germination rates of spinach seeds

Lowering the initial moisture index from 0.75 to 0.33 (corresponding to an increase in the initial substrate-to-water ratio from 1:3 to 1:0.5) increased the spinach seed visible germination rate from 23 % to 69 % for 'Corvair', from 35 % to 87 % for 'Kolibri', from 43 % to 85 % for 'Seaside', and from 27 % to 86 % for 'Space' (Fig. 1). The moisture indices of 0.67 and 0.75 resulted in similarly low germination rates. In contrast, the moisture index of 0.50 resulted in moderately improved germination, while the moisture index of 0.33 resulted in the highest germination rates. At each moisture index, 'Corvair' exhibited the lowest visible germination rate among the four cultivars (Fig. 1).

3.2. Individual shoot fresh mass and leaf morphology

Because the initial moisture index either did not influence ($P > 0.05$) or minimally influenced individual shoot fresh mass and leaf morphology at harvest, data of these parameters were grouped across initial moisture indices and analyzed based on the nutrient solution nitrogen concentration without and with active aeration (Fig. 2). Active aeration increased the shoot fresh mass of individual plants by 68 %–102 % for 'Corvair', 59 %–143 % for 'Kolibri', 72 %–104 % for 'Seaside', and 63 %–94 % for 'Space' across all nutrient solution nitrogen concentrations when compared to no active aeration (Fig. 2A–D). Active aeration increased the leaf length of individual plants by 24 %–37 % for 'Corvair', 25 %–54 % for 'Kolibri', 30 %–44 % for 'Seaside', and 31 %–49 % for 'Space' across all nutrient solution nitrogen concentrations when compared to no active aeration (Fig. 2E–H). Active aeration increased the leaf width of individual plants by 35 %–41 % for 'Corvair', 21 %–61 % for 'Kolibri', 31 %–39 % for 'Seaside', and 34 %–49 % for 'Space' across all nutrient solution nitrogen concentrations when compared to no active aeration (Fig. 2I–L).

Without active aeration, the nutrient solution nitrogen concentration did not affect individual shoot fresh mass or leaf length and width of

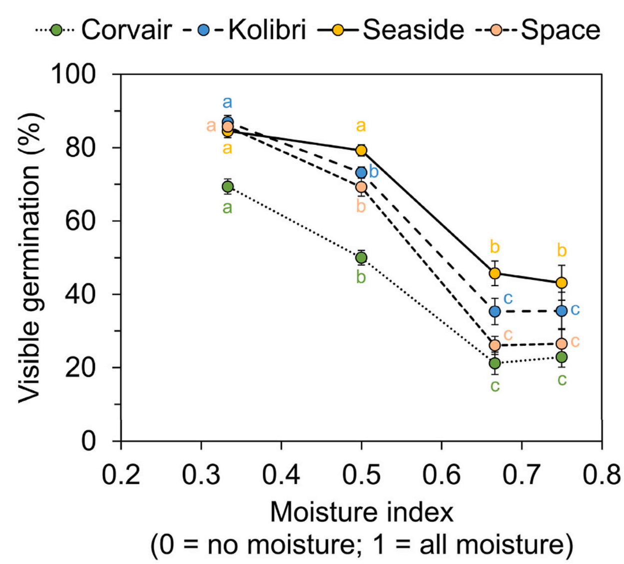

Fig. 1. Visible germination percentages of four spinach cultivars ('Corvair', 'Kolibri', 'Seaside', and 'Space') in foam trays filled with a pre-moistened peat-perlite substrate at four initial substrate-to-water ratios (1:0.5, 1:1, 1:2, and 1:3, w/w), which corresponded to four moisture indices [$weight_{water} \div (weight_{water} + weight_{substrate})$] of 0.33, 0.50, 0.67, and 0.75, respectively. The moisture index ranges from 0 (when no added moisture is present in the substrate) to 1 (when there is only moisture, without any substrate). Means (±SE) followed by different letters within each cultivar are statistically different based on Tukey's honest significant difference test ($P \leq 0.05$).

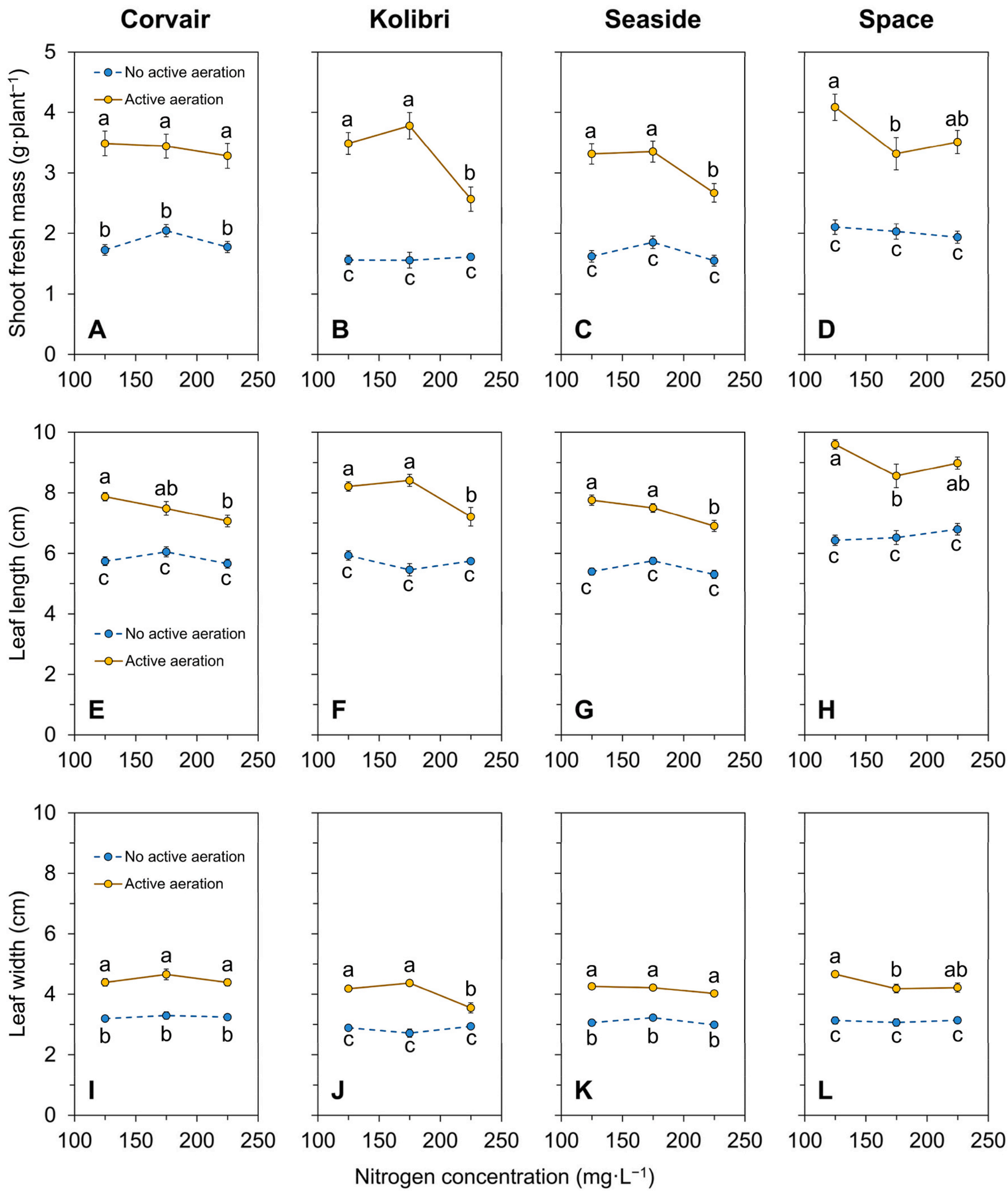

Fig. 2. The (**A–D**) individual shoot fresh mass and the (**E–H**) length and (**I–L**) width of the largest leaf per plant of four spinach cultivars ('Corvair', 'Kolibri', 'Seaside', and 'Space') grown in a greenhouse as baby greens in deep-water-culture food tables under six nutrient solution treatments, including three nutrient solution nitrogen concentrations (125, 175, and 225 mg L^{-1}), each without (blue scatters and dashed lines) and with (yellow scatters and solid lines) active aeration. Means (±SE) followed by different letters within each cultivar and parameter are statistically different based on Tukey's honest significant difference test ($P \leq 0.05$).

baby spinach plants (Fig. 2). With active aeration, increasing the nutrient solution nitrogen concentration from 125 to 175 mg L^{-1} did not consistently influence these parameters for most cultivars. For 'Space', growth tended to decline with higher nitrogen concentration, showing 19 % lower shoot fresh mass and 10 %–11 % lower leaf length and width at 175 mg L^{-1} compared to 125 mg L^{-1} (Fig. 2D, H, and 2L). Further increasing the nutrient solution nitrogen concentration to 225 mg L^{-1} did not cause additional reduction in 'Space' and did not influence growth in 'Corvair', but decreased shoot fresh mass of 'Kolibri' and 'Seaside' by 20 %–32 % (Fig. 2).

3.3. Total shoot fresh mass and chlorophyll index

Because the nutrient solution nitrogen concentration did not influence ($P > 0.05$) total shoot fresh mass or the leaf chlorophyll index at harvest, data of these parameters were grouped across nutrient solution nitrogen concentrations and analyzed based on the initial moisture index without and with active aeration (Fig. 3). The shoot fresh mass of all four cultivars responded to the initial moisture index similarly to the visible germination rate. Decreasing the initial moisture index from 0.75 to 0.33 (i.e., from right to left along the x-axis in Fig. 3) increased the total shoot fresh mass per tray quadrant by 79 %–199 % without active

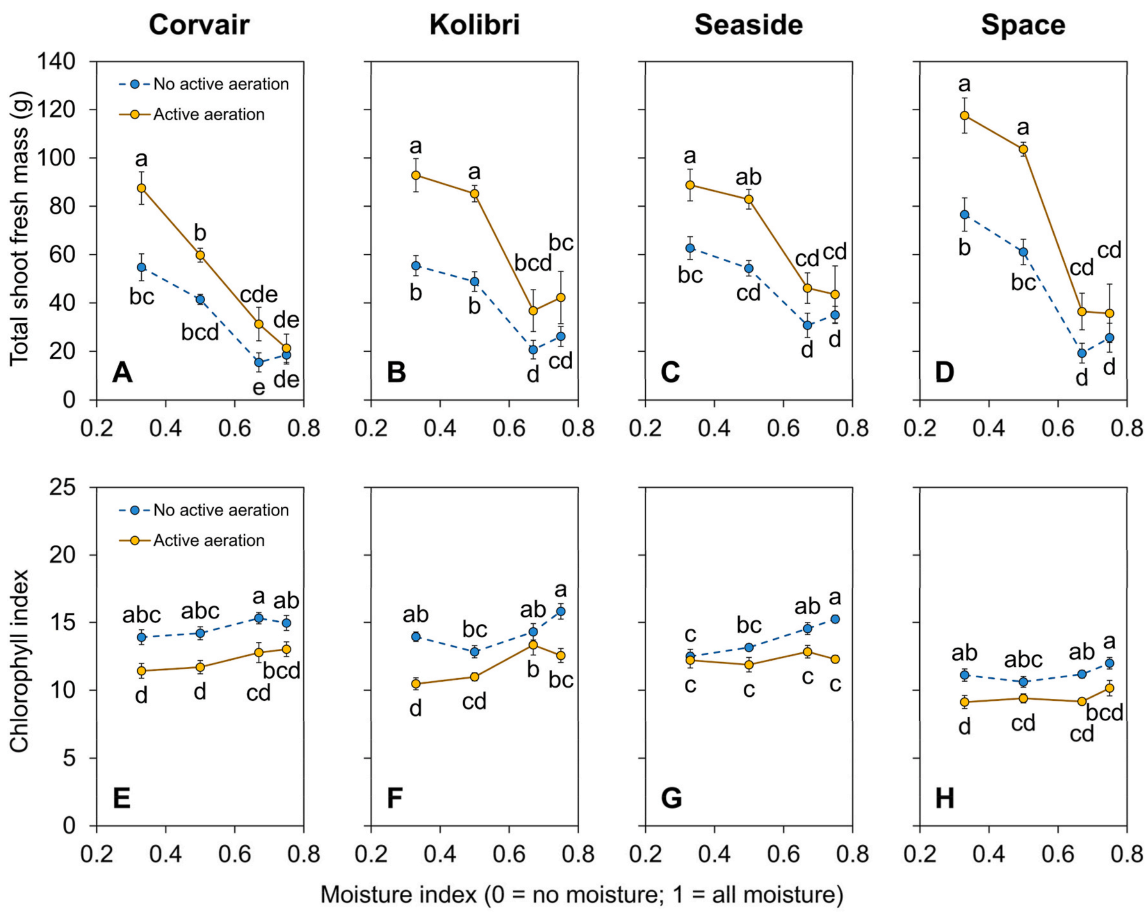

Fig. 3. The (**A–D**) total shoot fresh mass per tray quadrant and the (**E–H**) chlorophyll index of the largest leaf per plant of four spinach cultivars ('Corvair', 'Kolibri', 'Seaside', and 'Space') grown in a greenhouse as baby greens in deep-water-culture flood tables under four initial substrate-to-water ratios (1:0.5, 1:1, 1:2, and 1:3, w/w), which corresponded to four moisture indices [$weight_{water} \div (weight_{water} + weight_{substrate})$] of 0.33, 0.50, 0.67, and 0.75, respectively. The moisture index ranges from 0 (when no added moisture is present in the substrate) to 1 (when there is only moisture, without any substrate). Plants receiving each moisture ratio were subsequently grown hydroponically without (blue scatters and dashed lines) and with (yellow scatters and solid lines) active aeration. Means (±SE) followed by different letters within each cultivar and parameter are statistically different based on Tukey's honest significant difference test ($P \leq 0.05$).

aeration and by 104 %–311 % with active aeration (Fig. 3A–D). For each cultivar, the magnitude of the increase was larger with active aeration than without. Decreasing the initial moisture index from 0.75 to 0.33 (i.e., from right to left along the x-axis in Fig. 3) did not influence the chlorophyll index of 'Corvair' or 'Space', irrespective of active aeration, but decreased that of 'Kolibri' with active aeration and that of 'Seaside' without active aeration both by 17 %–18 % (Fig. 3E–H).

For all four cultivars, active aeration generally increased the total shoot fresh mass per tray quadrant by 42 %–74 % at the lower initial moisture indices of 0.33 and 0.50 when compared to no active aeration. In contrast, active aeration did not influence the total shoot fresh mass per tray quadrant at the higher initial moisture indices of 0.67 and 0.75 (Fig. 3A–D). Active aeration decreased the chlorophyll index of all four cultivars when compared to no active aeration; however, there were exceptions at certain cultivar-specific initial moisture indices (Fig. 3E–H). When active aeration was analyzed as a single factor, it decreased the chlorophyll index of all four cultivars by 11 %–17 % (Fig. 4).

4. Discussion

In this study, lowering the moisture index from 0.75 to 0.33 markedly improved spinach seed germination in a peat-perlite substrate for all four cultivars, highlighting the sensitivity of spinach seeds to moisture and the pivotal role of moisture control in germination. Inhibition of spinach seed germination occurs in a wet root-zone environment, which impedes the required oxygen supply to the seed embryo [8,23]. The amount of water in soil can be quantified by the soil water potential. With the potential of free water being 0, drier soils have more negative soil water potentials. Spinach seed germination is inhibited when the soil water potential exceeds −500 mm (wetter) but promoted when the soil water potential falls below −500 mm (drier) [23], which is consistent with results in this study. High moisture is not an issue in natural non-waterlogged field conditions, where the soil water potential is generally below −500 mm [24]. However, controlled-environment spinach growers who mix soilless substrates with water need to exercise care in moisture control. Moisture indices below 0.33 and above 0.75 produced highly dry and waterlogged conditions, respectively, which would not be conducive to germination. However, moisture indices below 0.33 should be tested in future research to identify the optimal moisture index.

The highest germination rate in a peat-based substrate in this study was under a moisture index of 0.33, which was between the moisture indices of 0.10 and 0.75 in two existing protocols [4,9]. The differences in optimal moisture indices reported in previous studies [4,9] are likely due to variations in the inherent moisture content or composition of the peat-based substrates used in those studies. In our experiment, all trays started with the same substrate, and the treatment differences were imposed by adding precise amounts of water to achieve the target moisture indices. Thus, the observed differences in germination within this study reflect the imposed moisture levels rather than any variation in initial substrate moisture. The observed germination trends may also

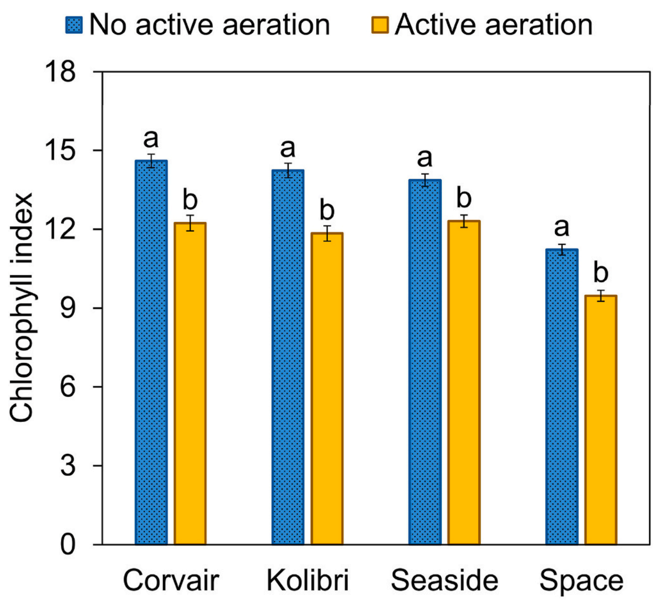

Fig. 4. The chlorophyll index of the largest leaf per plant of four spinach cultivars ('Corvair', 'Kolibri', 'Seaside', and 'Space') grown in a greenhouse as baby greens in deep-water-culture flood tables without (dot-filled blue bars) and with (blank yellow bars) active aeration. Data were analyzed with active aeration as a single factor to show its effects. Means (±SE) followed by different letters within each cultivar are statistically different based on Tukey's honest significant difference test ($P \leq 0.05$).

have been influenced by improved oxygen diffusion in moderately moist substrates, which facilitates embryo respiration [25]. Additionally, reduced pathogen pressure under well-aerated conditions may have contributed [26], although no pathogen-related seedling failure was observed across treatments. Growers are recommended to identify their own optimal moisture index using the methods described in this study. In this study, decreasing the initial moisture index from 0.75 to 0.33 increased the total shoot fresh mass per tray quadrant for all four spinach cultivars following similar trends to germination rates. Given that individual shoot growth was minimally affected by the moisture index, higher germination rates under lower moisture indices led to more plants and thus higher total harvestable yield.

Active aeration increased leaf length, leaf width, and shoot fresh mass in baby spinach. In hydroponic systems, plant roots fully submerged in water can suffer from oxygen deficiency without proper active aeration. Active aeration improves the availability of dissolved oxygen crucial for root respiration as well as for nutrient and water uptake [27–29]. The impact of active aeration on hydroponic plant growth is generally more significant under conditions of dissolved oxygen deficiency. For example, as dissolved oxygen levels naturally decreased with higher temperatures, oxygen supplementation had a greater impact on photosynthesis and plant dry mass of spinach in warmer conditions (33 vs. 20 °C) [11]. Similarly, as dissolved oxygen levels declined over time during the growing cycle, longer cycles increased the extent and duration of oxygen deficiency, making oxygen supplementation more beneficial for crop yield during extended crop cycles [29]. In this study, despite relatively cool conditions (air temperature of 21 °C) and a short growing period (15 d), active aeration still increased leaf size and shoot fresh mass in all four spinach cultivars. This was likely due to the high planting density (2268 plants·m^{-2}) in substrate-filled expanded polystyrene trays, a common practice in hydroponic baby spinach production, where oxygen competition in the root zone can occur. Additionally, the peat-based substrate can become waterlogged in the tray due to its constant contact with the nutrient solution in deep-water culture, limiting the natural diffusion of oxygen and amplifying the benefits of supplemental dissolved oxygen in supporting root function and overall plant growth.

For the individual shoot fresh mass and leaf morphology of all four spinach cultivars, effects of the nutrient solution nitrogen concentration depended on active aeration. Without active aeration, the nutrient solution nitrogen concentration did not influence baby spinach biomass and leaf morphology. However, with active aeration, increasing the nutrient solution nitrogen concentration from 125 to 225 mg L^{-1} decreased these parameters. This interaction may be attributed to the active transport of nitrogen in roots. Root uptake of nitrate nitrogen is an active process facilitated by specialized protein transporters in the NPF and NRT2 families on root hairs [30]. The process of nitrate nitrogen absorption and assimilation into cells requires energy, which is generated from root respiration [31,32]. Because root respiration requires oxygen, active aeration of the nutrient solution increased the dissolved oxygen level, which likely increased energy production from root respiration and promoted nitrate nitrogen uptake. Higher-than-optimal nitrogen application rates reduced the growth of soil-grown spinach 'Ohio' and 'Quanneng' compared to intermediate optimal rates [33,34]. Therefore, at higher nutrient solution nitrogen concentrations of 175 and 225 mg L^{-1} in this study, the increased nitrate nitrogen uptake with active aeration could be excessive and cause symptoms of nitrogen toxicity such as stunted growth. Adequate nitrogen is critical to maximize spinach growth as increasing the nutrient solution nitrogen concentration from 13 to 105 mg L^{-1} increased leaf biomass of spinach 'Melody' and 'Springer F1' [35]. Our results suggest that 125 mg L^{-1} of nitrogen was sufficient to achieve high nitrogen use efficiency and plant growth rates, with no benefits above this threshold, in greenhouse hydroponic baby spinach production.

Active aeration decreased the chlorophyll index in all four spinach cultivars. Chlorophyll index tends to decrease during oxygen deprivation [36], and research has shown that active aeration can increase the chlorophyll index [14,37,38]. For example, increasing the dissolved oxygen level from 1.8–3.8 to 5.3 mg L^{-1} through active aeration increased the chlorophyll index of pepper 'California Wonder' under ammonium-based fertilization [14]. The increase in the chlorophyll index under active aeration has been at least partially attributed to increased chlorophyll accumulation driven by improved nitrogen uptake under higher dissolved oxygen conditions [14,37]. In this study, however, the nutrient solution nitrogen concentrations between 125 and 225 mg L^{-1} were all sufficient for baby spinach growth, suggesting that nitrogen was not a limiting factor, so active aeration might not improve nitrogen uptake and thus chlorophyll accumulation. Moreover, the chlorophyll index is a relative indicator of chlorophyll concentration, which is chlorophyll content per unit leaf area [39]. Because active aeration increased leaf length and width, leading to larger overall leaf areas, the observed decrease in the chlorophyll index under active aeration was likely due to a dilution effect caused by the expanded leaf area, rather than an absolute reduction in total chlorophyll content within the plant. It is possible that the combination of supplemental lighting and active aeration might have increased oxidative stress (reactive oxygen species) in plants under some conditions [40], which could affect chlorophyll synthesis. However, reactive oxygen species were not measured in this study, and the observed decrease in the chlorophyll index was most likely due to the dilution effect from expanded leaf area.

In conclusion, a lower moisture index of 0.33 (or a higher substrate-to-water ratio of 1:0.5, w/w) in peat-perlite media markedly improved seed germination of the four spinach cultivars, without any seed treatment, compared to higher moisture indices (or lower substrate-to-water ratios). Subsequently, higher germination rates under lower initial moisture indices translated to higher total shoot biomass. After spinach seedlings were floated in greenhouse deep-water culture, active aeration of the nutrient solution increased harvestable shoot biomass and extension growth of hydroponic baby spinach. During the production period, increasing the nutrient solution nitrogen concentration beyond 125 mg L^{-1} did not benefit baby spinach growth. Therefore, it is recommended to use active aeration and moderately low nitrogen when growing greenhouse hydroponic baby spinach to maximize crop growth and nutrient use efficiency.

CRediT authorship contribution statement

Qingwu Meng: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kishan Biradar:** Writing – review & editing, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Yujin Park:** Writing – review & editing, Writing – original draft. **Patrick N. Kelley:** Writing – review & editing, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Qingwu Meng reports financial support was provided by US Department of Agriculture. Qingwu Meng reports equipment, drugs, or supplies was provided by JR Peters, Inc. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding for this research was provided by the Innovations in Plant Science Graduate Fellowship in the Department of Plant and Soil Sciences at the University of Delaware. This work was also supported by the Urban, Indoor, and Emerging Agriculture Program (project award no. 2023-70019-39371) from the U.S. Department of Agriculture's National Institute of Food and Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy. We thank University of Delaware greenhouse staff and undergraduate students for experimental assistance and JR Peters, Inc. for fertilizer support.

Data availability

Data will be made available on request.

References

[1] M.A. Murcia, A.M. Jiménez-Monreal, J. Gonzalez, M. Martínez-Tomé, Spinach, in: A.K. Jaiswal (Ed.), Nutritional Composition and Antioxidant Properties of Fruits and Vegetables, Academic Press, Cambridge, MA, USA, 2020, pp. 181–195, https://doi.org/10.1016/B978-0-12-812780-3.00011-8.

[2] T. Shelford, The Risk of *Pythium aphanidermatum* in Hydroponic baby-leaf Spinach Production (Phd Diss), Cornell University, Ithaca, New York, United States, 2010. https://hdl.handle.net/1813/17229.

[3] N. Mattson, Pythium root rot on hydroponically grown basil and spinach, e-GRO Edible Alert 3 (1) (2018) 1–5. https://e-gro.org/pdf/E301.pdf.

[4] M. Brechner, D. de Villiers, Cornell Controlled Environment Agriculture Hydroponic Spinach Production Handbook, Cornell University CEA Program, 2013. https://bpb-us-e1.wpmucdn.com/blogs.cornell.edu/dist/8/8824/files/2019/06/Cornell-CEA-baby-spinach-handbook.pdf.

[5] H.B. Sifton, On the germination of the seed of *Spinacia oleracea*, L., at low temperatures, Ann. Bot. 41 (163) (1927) 557–568. https://www.jstor.org/stable/43237080.

[6] L.S. Katzman, A.G. Taylor, R.W. Langhans, Seed enhancements to improve spinach germination, Hortscience 36 (5) (2001) 979–981, https://doi.org/10.21273/HORTSCI.36.5.979.

[7] N.J. Langenfeld, B. Bugbee, Germination and seedling establishment for hydroponics: the benefit of slant boards, PLoS One 17 (10) (2022) e0275710, https://doi.org/10.1371/journal.pone.0275710.

[8] K.J. Magnée, O.E. Scholten, J. Postma, E.T. Lammerts van Bueren, S.P. Groot, Sensitivity of spinach seed germination to moisture is driven by oxygen availability and influenced by seed size and pericarp, Seed Sci. Technol. 48 (1) (2020) 117–131, https://doi.org/10.15258/sst.2020.48.1.13.

[9] C. Kubota, S. Offenbecher, R. Conrad, Pericarp cracking and moisture management to improve spinach germination, e-GRO Edible Alert. 8 (12) (2023) 1–5. https://www.e-gro.com/pdf/E812.pdf.

[10] C. Chun, T. Takakura, Rate of root respiration of lettuce under various dissolved oxygen concentrations in hydroponics, Environ. Control Biol. 32 (2) (1994) 125–135, https://doi.org/10.2525/ecb1963.32.125.

[11] T. Morimoto, T. Masuda, H. Nonami, Oxygen enrichment in deep hydroponic culture improves growth of spinach, Environ. Control Biol. 27 (3) (1989) 97–102, https://doi.org/10.2525/ecb1963.27.97.

[12] H. Ikeura, H. Takahashi, F. Kobayashi, M. Sato, M. Tamaki, Effects of microbubble generation methods and dissolved oxygen concentrations on growth of Japanese mustard spinach in hydroponic culture, J. Hortic. Sci. Biotechnol. 93 (5) (2018) 483–490, https://doi.org/10.1080/14620316.2017.1391718.

[13] E. Goto, A.J. Both, L.D. Albright, R.W. Langhans, A.R. Leed, Effect of dissolved oxygen concentration on lettuce growth in floating hydroponics, Acta Hortic. 440 (1996) 205–210, https://doi.org/10.17660/ActaHortic.1996.440.36.

[14] H.R. Roosta, The responses of pepper plants to nitrogen form and dissolved oxygen concentration of nutrient solution in hydroponics, BMC Plant Biol. 24 (1) (2024) 281, https://doi.org/10.1186/s12870-024-04943-7.

[15] J. Li, P. Yang, H. Sohail, H. Du, J. Li, The impact of short-term nitrogen starvation and replenishment on the nitrate metabolism of hydroponically grown spinach, Sci. Hortic. 309 (2023) 111632, https://doi.org/10.1016/j.scienta.2022.111632.

[16] X. Liu, B. Hu, C. Chu, Nitrogen assimilation in plants: current status and future prospects, J. Genet. Genomics. 49 (5) (2022) 394–404, https://doi.org/10.1016/j.jgg.2021.12.006.

[17] D. Djidonou, D.I. Leskovar, Seasonal changes in growth, nitrogen nutrition, and yield of hydroponic lettuce, Hortscience 54 (1) (2019) 76–85, https://doi.org/10.21273/HORTSCI13567-18.

[18] N.S. Mattson, C. Peters, A recipe for hydroponic success, Grower 1 (2014) 16–19. https://bpb-us-e1.wpmucdn.com/blogs.cornell.edu/dist/b/5759/files/2015/03/hydroponic-recipes-1bms8vj.pdf.

[19] S. Nicola, C. Egea-Gilabert, D. Niñirola, E. Conesa, G. Pignata, E. Fontana, J. A. Fernández, Nitrogen and aeration levels of the nutrient solution in soilless cultivation systems as important growing conditions affecting inherent quality of baby leaf vegetables: a review, Acta Hortic. 1099 (2015) 167–177, https://doi.org/10.17660/ActaHortic.2015.1099.17.

[20] H.Y. Dasgan, S. Kacmaz, B.B. Arpaci, B. İkiz, N.S. Gruda, Biofertilizers improve the leaf quality of hydroponically grown baby spinach (*Spinacia oleracea* L.), Agronomy 13 (2) (2023) 575, https://doi.org/10.3390/agronomy13020575.

[21] D.P. Gillespie, G. Papio, C. Kubota, High nutrient concentrations of hydroponic solution can improve growth and nutrient uptake of spinach (*Spinacia oleracea* L.) grown in acidic nutrient solution, Hortscience 56 (6) (2021) 687–694, https://doi.org/10.21273/HORTSCI15777-21.

[22] C.P. Levine, N.S. Mattson, Potassium-deficient nutrient solution affects the yield, morphology, and tissue mineral elements for hydroponic baby leaf spinach (*Spinacia oleracea* L.), Horticulturae 7 (8) (2021) 213, https://doi.org/10.3390/horticulturae7080213.

[23] W. Heydecker, P.I. Orphanos, The effect of excess moisture on the germination of *Spinacia oleracea* L, Planta 83 (1968) 237–247, https://doi.org/10.1007/BF00385333.

[24] P.J. Salter, J.B. Williams, The influence of texture on the moisture characteristics of soils: I. a Critical comparison of techniques for determining the available-water capacity and moisture characteristic curve of a soil, J. Soil Sci. 16 (1) (1965) 1–15, https://doi.org/10.1111/j.1365-2389.1965.tb01416.x.

[25] S. Dasberg, K. Mendel, The effect of soil water and aeration on seed germination, J. Exp. Bot. 22 (4) (1971) 992–998, https://doi.org/10.1093/jxb/22.4.992.

[26] I. Martín, L. Gálvez, L. Guasch, D. Palmero, Fungal pathogens and seed storage in the dry state, Plants 11 (22) (2022) 3167, https://doi.org/10.3390/plants11223167.

[27] D. Alvarado-Camarillo, L.A. Valdez-Aguilar, J.A. González-Fuentes, E. Rascón-Alvarado, F.M. Peña-Ramos, Response of hydroponic lettuce to aeration, nitrate and potassium in the nutrient solution, Acta Agric. Scandinavica, Section B—Soil Plant Sci. 70 (4) (2020) 341–348, https://doi.org/10.1080/09064710.2020.1730430.

[28] B. Baiyin, K. Tagawa, M. Yamada, X. Wang, S. Yamada, S. Yamamoto, Y. Ibaraki, Study on plant growth and nutrient uptake under different aeration intensity in hydroponics with the application of particle image velocimetry, Agriculture 11 (11) (2021) 1140, https://doi.org/10.3390/agriculture11111140.

[29] A. Lenzi, A. Baldi, R. Tesi, Growing spinach in a floating system with different volumes of aerated or non-aerated nutrient solution, Adv. Hortic. Sci. 25 (1) (2011) 21–25, https://doi.org/10.13128/ahs-12780.

[30] C. Muratore, L. Espen, B. Prinsi, Nitrogen uptake in plants: the plasma membrane root transport systems from a physiological and proteomic perspective, Plants 10 (4) (2021) 681, https://doi.org/10.3390/plants10040681.

[31] A.J. Bloom, S.S. Sukrapanna, R.L. Warner, Root respiration associated with ammonium and nitrate absorption and assimilation by barley, Plant Physiol. 99 (4) (1992) 1294–1301, https://doi.org/10.1104/pp.99.4.1294.

[32] N.M. Crawford, A.D. Glass, Molecular and physiological aspects of nitrate uptake in plants, Trends Plant Sci. 3 (10) (1998) 389–395, https://doi.org/10.1016/S1360-1385(98)01311-9.

[33] L.E. Nemadodzi, H. Araya, M. Nkomo, W. Ngezimana, N.F. Mudau, Nitrogen, phosphorus, and potassium effects on the physiology and biomass yield of baby spinach (*Spinacia oleracea* L.), J. Plant Nutr. 40 (14) (2017) 2033–2044, https://doi.org/10.1080/01904167.2017.1346121.

[34] J. Zhang, Z. Sha, Y. Zhang, Z. Bei, L. Cao, The effects of different water and nitrogen levels on yield, water and nitrogen utilization efficiencies of spinach

(*Spinacia oleracea* L.), Can. J. Plant Sci. 95 (4) (2015) 671–679, https://doi.org/10.4141/cjps-2014-184.
[35] M.G. Lefsrud, D.A. Kopsell, D.E. Kopsell, Nitrogen levels influence biomass, elemental accumulations, and pigment concentrations in spinach, J. Plant Nutr. 30 (2) (2007) 171–185, https://doi.org/10.1080/01904160601117838.
[36] J. Ma, G. Rukh, Z. Ruan, X. Xie, Z. Ye, D. Liu, Effects of hypoxia stress on growth, root respiration, and metabolism of *Phyllostachys praecox*, Life 12 (6) (2022) 808, https://doi.org/10.3390/life12060808.
[37] J.D. Butcher, C.P. Laubscher, J.C. Coetzee, A study of oxygenation techniques and the chlorophyll responses of *Pelargonium tomentosum* grown in deep water culture hydroponics, Hortscience 52 (7) (2017) 952–957, https://doi.org/10.21273/HORTSCI11707-16.
[38] R. Tesi, A. Lenzi, P. Lombardi, Effect of different O_2 levels on spinach (*Spinacia oleracea* L.) grown in a floating system, Acta Hortic. 614 (2002) 631–637, https://doi.org/10.17660/ActaHortic.2003.614.94.
[39] C. Parry, J.M. Blonquist Jr, B. Bugbee, In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship, Plant Cell Environ. 37 (11) (2014) 2508–2520, https://doi.org/10.1111/pce.12324.
[40] P. Borbély, A. Gasperl, T. Pálmai, M. Ahres, M.A. Asghar, G. Galiba, M. Müller, G. Kocsy, Light intensity-and spectrum-dependent redox regulation of plant metabolism, Antioxidants 11 (7) (2022) 1311, https://doi.org/10.3390/antiox11071311.