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Research Paper

Light intensity management as a strategy to enhance folate accumulation in soilless baby leaf production

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ABSTRACT

Folate deficiency remains a relevant public health concern, and agronomic biofortification may represent a sustainable strategy to enhance dietary intake through fresh vegetables. This study evaluated the effect of different light intensity regimes on folate accumulation, yield, mineral composition, and nitrate content in mizuna (*Brassica rapa* L. var. japonica), pakchoi (*Brassica rapa* L. subsp. chinensis), and chicory (*Cichorium intybus* L.) grown in a soilless system under LED lighting. Three treatments were applied over a 30day growth cycle: a constant light intensity of 200 μmol m^{-2} s^{-1} (T1); an initial 23day period at 200 μmol m^{-2} s^{-1} followed by a 7day increase to 300 μmol m^{-2} s^{-1} (T2); and a constant light intensity of 300 μmol m^{-2} s^{-1} (T3).

Folate content significantly increased under T3 (+24% vs. T1 and T2), particularly in pakchoi, without affecting fresh yield. Higher light intensity also reduced NO_3^- accumulation (up to −80% in pakchoi), whereas no substantial changes were observed in mineral composition. A 100 g portion of mizuna (T2) or pakchoi (T3) can provide up to 45% of the adult Recommended Daily Allowance (RDA) for folate. Targeted PPFD management therefore represents a promising chemical-free strategy to enhance micronutrient density, although species and genotype selection remain crucial for effective biofortification.

1. Introduction

A proper diet, along with adequate physical activity and the control of different risk factors, such as alcohol consumption and/or smoking, is an important element in preventing many diseases. It has indeed been demonstrated that dietary choices and lifestyle play a significant role in the ability to avoid the development of diseases, control their progression, or, conversely, trigger their onset.

A balanced diet thus serves as an effective preventive strategy. Furthermore, particular attention should be given to meeting the Recommended Daily Allowance (RDA) of micronutrients such as iodine, zinc, iron, and various groups of vitamins (A, B, C, and K), whose deficiency can induce or promote the development of various pathologies (Tulchinsky et al., 2010). Under specific physiological conditions, such as during pregnancy and breastfeeding, and in particular socioeconomic contexts, the intake of specific nutrients through diet may not be sufficient to meet nutritional needs, potentially leading to detrimental conditions (Stamm et al., 2013; Jouanne et al., 2021; Volkert et al., 2004). Today, it is possible to prevent numerous diseases through preventive nutritional approaches, such as the consumption of fortified and/or biofortified foods (Crider et al., 2011; Buturi et al., 2021).

Among nutritional deficiencies in micronutrients, folate deficiency is one of the most important from a health perspective (Infante-Rivard et al., 1986). Although folate deficiency is more common in low-income countries, it is also true that this deficiency is present in industrialized countries; it occurs in specific population groups such as the elderly and pregnant women (Stamm et al., 2013). Additionally, this deficiency can be caused by inadequate dietary intake or increased requirements (e.g., during pregnancy and breastfeeding), impaired absorption and metabolism, or the use of certain medications or excessive alcohol consumption (Brouwer et al., 2001).

The daily requirement varies depending on physiological conditions and age; in a healthy adult, it is 400 μg/day, while during pregnancy and lactation, it is 600 μg/day and 500 μg/day, respectively (Xu et al., 2022; Bekaert et al., 2008). Folate is naturally found in different foods, such as vegetables, fruits, fruit juices, nuts, legumes, seafood, eggs, dairy

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products, meat, poultry, and cereals (Witthöft et al., 1999). Leafy vegetables can show different folate content depending on species and/or genotypes (Kołton et al. 2022), and, most likely, also depending on the chlorophyll content, since the synthesis of folate and chlorophyll share part of the metabolic pathway (their synthesis seems to be correlated through the methyl cycle) (Van Wilder et al., 2009; Shohag et al., 2020, 2012). In the horticultural sector, leafy vegetables known as "baby leaf" are gaining popularity among consumers worldwide because they represent a rich source of minerals, vitamins, including folates, and other phytochemicals with important health effects (Di Gioia et al., 2017). The nutritional potential of baby leaf may be due to a higher concentration of metabolites associated with a higher dry matter content compared to mature leaves (Martínez-Ispizua et al., 2022). Furthermore, the biochemical changes occurring during germination, responsible for the higher levels of bioactive compounds in sprouts and microgreens, may further influence the nutritional quality of baby leaf vegetables (Di Gioia et al., 2017). The higher nutritional value of baby leaf vegetables has been found in different genotypes and for different metabolites (Bergquist et al., 2005; Martínez-Ispizua et al., 2022; Mallor et al., 2023).

Recent studies have focused on the use of LED lighting in soilless cultivation systems to optimize photosynthetic processes, thereby improving the agronomic performance and the nutritional value of vegetables products (Demir et al., 2023; Guiamba et al., 2022). The use of calibrated lights, based on specific light intensity and spectral composition, allowed a significant 50% increase in mean fresh weight in all species and for all spectra studied (Nájera and Urrestarazu, 2019). From a nutritional quality perspective, results are variable depending on genotypes and metabolites. In mizuna, only magnesium and zeaxanthin contents increased at increasing light intensity from 200 to 800 μmol m^{-2} s^{-1}; other bioactive compounds, such as anthocyanins, β-carotene, lutein, ascorbic acid, and thiamine, showed steady or decreasing trends but mainly due to a dilution effect corresponding to greater dry mass production with increasing light intensity (Darby et al., 2025). By varying the LED light spectra, it is possible to modulate the biosynthesis of different bioactive compounds, such as the total and individual glucosinolate content, achieving the highest glucoraphanin content in broccoli microgreens under blue light or the highest glucoraphanin and total glucosinolate content with red+blue light in cabbage, while in radish microgreens the highest glucosinolate and glucoraphasatin contents were found with red, blue, far red and red+far read LED (Demir et al., 2023). LED light has been shown to have an impact on folate biosynthesis (Gao et al., 2023) and NO_3^- content (Nájera and Urrestarazu, 2019). Gao et al. (2023) reported that the application of white light LED (10 μmol m^{-2} s^{-1}) allowed an increased folate content in pakchoi during storage. Besides light, few studies have investigated the influence of cultivation or environmental factors on folate biosynthesis in green vegetables (Kołton et al., 2022). Most attempts to increase folate synthesis in plants rely on metabolic engineering methods, generally applied to staple and some other species, resulting in biofortified crops (Saini et al., 2016). An alternative strategy relies on non-genetic biofortification, achieved either by supplementing fertilizers with enhancers such as glutamic acid, phenylalanine, or magnesium—though with variable outcomes (Watanabe et al., 2017)—or through foliar applications of folate synthesis inducers, including phenylalanine or salicylic acid (Bonasia et al., 2025).

In light of the growing interest in promising strategies to enhance the nutritional quality of fresh vegetables, this study aimed to evaluate the effectiveness of an innovative lighting management strategy, based on increased photosynthetic photon flux density (PPFD), in enhancing folate accumulation in three baby leaf species—pakchoi (*Brassica rapa* L. subsp. *chinensis*), mizuna (*Brassica rapa* L. var. *japonica*), and chicory (*Cichorium intybus* L.), cultivated in a controlled soilless system. Kołton et al. (2022) suggest that although research on light's influence on plant folate synthesis yields mixed results, distinct correlations with light stimuli are evident. However, the wide range of species-specific reactions warrants careful attention.

Specifically, the objective was to determine whether a constant or late-cycle increase in light intensity could promote folate biosynthesis in the edible portion without negatively affecting crop yield and mineral profile, while also monitoring nitrate concentration. The working hypothesis was that exposure to higher PPFD levels, particularly during the final phase of the cultivation cycle, would stimulate folate biosynthesis through the enhancement of light-dependent metabolic pathways, leading to a significant increase in folate concentration in these species, without compromising agronomic performance included the Energy consumption for plant production or food safety parameters.

2. Materials and methods

2.1. Plant materials and experimental conditions

The experiment was conducted in a walk-in growth chamber located at the Institute of Sciences of Food Production (ISPA-CNR) in Bari (BA), with internal dimensions of 260×284×253 cm (length, width, and height, respectively). The plants were cultivated with a photoperiod of 14 hours of light and 10 hours of darkness. The mean air temperature and relative humidity inside the growth chamber during the experiment were 22/16 °C and 65/55%, respectively, for day and night.

The seeds of pakchoi (*Brassica rapa L.* subsp. *chinensis*; purchased from Riccardo Larosa sementi), mizuna (*Brassica rapa L.* var. *nipposinica*; purchased from Riccardo Larosa sementi), and chicory (*Cichorium intybus* L.; purchased from FuSeeds) were sown in cell pots containing peat.

To ensure seed germination, the cell pots were placed under carefully controlled conditions with a temperature of 22 °C and a humidity level of 90%. The CO_2 concentration within the growth chamber was maintained at ambient atmospheric levels through a continuous air exchange system, which ensured constant renewal of the internal atmosphere and prevented CO_2 depletion or accumulation throughout the experimental period. The CO_2 levels were periodically monitored and ranged between 500 and 530 ppm, corresponding to typical environmental concentrations. No supplemental CO_2 enrichment was applied. After seven days from sowing, when the seedlings reached the two-true-leaves stage, they were carefully transferred to a floating hydroponic system, where they were grown until the end of the experimental period. In this system, the plants (500 plant/m^2) are placed on a floating polystyrene support, with their roots in direct contact with the nutrient solution. The growth period lasted 30 days. The vegetables were cultivated using a nutrient solution (NS) prepared with distilled water. The NS was composed of N (140 mg L^{-1}), P (50 mg L^{-1}), K (200 mg L^{-1}), Mg (40 mg L^{-1}), Ca (100 mg L^{-1}), and S (69 mg L^{-1}). Micronutrients were added according to the NS proposed by D'Imperio et al. (2016). The electrical conductivity (EC) of the nutrient solution was 1.8 dS m^{-2}, and the pH was 5.8. EC and pH values were monitored every two days, and at the same time, top-ups with fresh nutrient solution were carried out to keep the values constant. A randomized complete block design with three replicates was adopted. The treatments were distributed across three distinct growing shelves, each shielded to prevent light contamination. On each shelf, three independent floating trays were placed, one for each species. Each tray contained 40 plants and was equipped with its own aeration system. The replicates were repeated over time while maintaining the same experimental settings for temperature and humidity. Throughout the experiment, the pH of the NS was measured every two days and adjusted to 5.5-6.0 using 1 M H_2SO_4. To ensure adequate oxygen supply and prevent potential root anoxia issues, an air pump was used in the hydroponic system. Three different light treatments were applied to baby leaf crops throughout the entire cultivation period (days 0–30). The first treatment (T1) consisted of constant light intensity at 200 μmol m^{-2} s^{-1}. The second treatment (T2) maintained the same intensity of 200 μmol m^{-2} s^{-1} until day 23, after which it was increased to 300 μmol m^{-2} s^{-1} for the remaining 7 days. The third treatment (T3) provided a constant light intensity of 300 μmol m^{-2} s^{-1} from seedling emergence to harvest, major

details were reported in Table 1S. The light intensity was periodically measured using a portable spectrometer (model Li-180, LI-COR Biosciences), and adjusted according to the plant's morphological growth, so as to ensure a constant light supply over time The baseline of 200 μmol m^{-2} s^{-1} was selected as a standard value for leafy vegetable cultivation in controlled environments (Chen et al., 2025); the 300 μmol m^{-2} s^{-1} treatment represents a +50% increase capable of stimulating secondary metabolism without causing photoinhibition. The 7-day exposure in T2 corresponds to the pre-harvest phase and represents a reasonable compromise between metabolic induction and energy cost containment.

2.2. Yield, leaf area and dry matter

At the harvest, yield [expressed as kg of fresh weight (FW) m^{-2}] and leaf area were assessed. For each replication, yield was quantified on 40 plants, while leaf area was determined on a subset of 10 plants using an image analysis software (ImageJ, Fiji version 1.53e). The acquisition of images was performed using a smartphone camera with a resolution of 25 megapixel. The camera was positioned at a fixed distance of 1 meter from the surface. The leaf area measurement was evaluated using a non-destructive method, which involved directly photographing the leaves. For this purpose, the leaves were carefully placed on a black surface with a ruler for scale reference.

For each replication, fresh baby leaf samples were collected from 10 plants and maintained in a forced draft oven at 65 °C until constant weight for the measurement of dry weight (DW). An additional set of 30 baby leaf samples per replication was freeze-dried using a Freeze Dry System (LABCONCO 7754030, Kansas City, MI, USA). The freeze-dried material was then ground to 500 μm using a laboratory mill (IKA A11 basic, Germany) to obtain a homogeneous powder. The resulting powdered samples were kept separate per replication and used for subsequent nutritional analysis.

2.3. Minerals and anions analysis

For nitrate determination, ion exchange chromatography (Dionex DX120, Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector was used as reported by D'Imperio et al. (2016). Briefly, the anions were extracted from DW samples with 3.5 mM (Na_2CO_3) and 1 mM ($NaHCO_3$) for 30 min. After extraction, the samples were diluted and filtered by using a 0.45 μm filter followed by Dionex OnGuard IIP (Thermo Fisher Scientific, Waltham MA, USA). The resulting solutions were analysed by ion chromatography (IC-Dionex DX120, Dionex Corporation) with a conductivity detector by using an Ion-Pac AG14 pre-column and an IonPac AS14 separation column (Dionex Corporation).

For Al, B, Fe, Mn, Ca, K, Mg, P, Na, Pb, Mo, Cd, Cu, Cr and Zn determination, 0.3 g of dried samples were mineralized with 10 mL of 65% HNO_3 (Pure grade, Carlo Erba) in a closed-vessel microwave assisted digestion system (MARS 6, CEM Corporation, Matthews, NC, USA). The digestion procedure was performed in two steps: 15 min to reach 200 °C and 10 min maintained at 200 °C (power set at 900–1050 W; 800 psi). The digested solutions were cooled at room temperature. The mineralized samples were diluted with ultrapure water (Milli-Q® Millipore 18 MΩ/cm) and filtered through a 0.45 μm membrane filter. Elemental analysis was performed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; 5100 VDV, Agilent Technologies, Santa Clara, CA, USA). Ca, K, Mg, P, and Na were quantified in radial mode whereas Al, B, Fe, Mn, Pb, Mo, Cd, Cu, Cr and Zn were measured in axial mode as reported by D'Imperio et al. (2022).

2.4. Folate extraction and 5-methyltetrahydrofolate (5-MTHF) quantification by HPLC

Folate extraction for each plant species and light treatment was performed in triplicate on the freeze-dried composite sample representative of each biological replicate following the method described by Czarnowska-Kujawska et al. (2017) with minor modifications. Briefly, 30 mg of dry plant material was homogenized in liquid nitrogen and mixed with 5 mL of extraction buffer (0.1 M MES, pH 6.1, containing 1% w/v ascorbic acid and 0.1% v/v 2-mercaptoethanol). Samples were incubated at 100 °C for 15 min and then rapidly cooled on ice. Subsequently, α-amylase solution (1 mg mL^{-1} in water, approximately 5 mg) was added to hydrolyze starch in the sample matrix, and 65 μL of rat serum (Biowest, Nuaillé, France) was added to enzymatically deconjugate folate polyglutamates to their monoglutamyl forms. The predominant monoglutamyl folate released by this procedure is 5-methyltetrahydrofolate (5-MTHF), which was therefore selected as the target analyte. Samples were incubated at 37 °C for 4 h and enzymes were then inactivated by heating at 100 °C for 5 min, and samples were cooled on ice. The mixture was centrifuged at 4,500 × g for 30 min at 4 °C, and the supernatant was recovered and adjusted to pH 7.0.

Quantification of 5-MTHF was performed by HPLC, adapted from Jastrebova et al. (2003) with minor modifications, and using external calibration with a 5-MTHF disodium salt standard (≥88% (UV-vis), Sigma Aldrich, St. Louis, USA). Standard concentrations were corrected for the declared purity of the reference material and the results were expressed as μg of 5-MTHF per 100 g of fresh weight, using the dry matter content to convert from dry weight basis. Analyses were carried out using an Agilent 1100 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a reversed-phase C18 column (Luna C18(2), 5 μm, 100 Å, 250 × 4.6 mm; Phenomenex, Torrance, CA, USA) maintained at 25 °C. The injection volume was 50 μL, and the flow rate was 0.5 mL min^{-1}. Detection was performed by fluorescence, with excitation/emission wavelengths set at 290/360 nm and photomultiplier tube gain set at 13 as optimized for the instrument used.

The mobile phases consisted of solvent A (water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid). The gradient elution was programmed as follows: 0–3 min, 95% A and 5% B; 7 min, 90% A and 10% B; 13 min, 70% A and 30% B; 20–22 min, 5% A and 95% B; 23 min, 95% A and 5% B. The system was re-equilibrated to the initial conditions before the next injection.

2.5. Statistical analysis

Effects of different treatments were tested using two-way analysis of variance (ANOVA, n = 3 biological replicates per treatment) followed by means separation with Fisher's protected least significant difference (LSD) at $\alpha = 0.05$. The statistical software STATISTICA 10.0 (StatSoft, Tulsa, OK, USA) was used for the analysis. In addition, the daily intake and the percentage contribution to the recommended dietary allowance (RDA) for folate were calculated separately for each species based on treatment means and assuming a 100 g fresh weight serving size.

3. Results and discussion

The crop performance and qualitative parameters of the three baby leaf species (mizuna, pakchoi, and chicory) showed significant differences depending on both the species and the applied light treatments, as reported in Table 1. Regarding yield (kg/m^2), the highest value was recorded for pakchoi, which was significantly higher than mizuna and chicory (Table 2). These data confirm that pakchoi has a greater capacity for biomass accumulation than mizuna and chicory, in line with previous observations on leafy brassicas (Li et al., 2026). However, the limited effect of light on fresh weight accumulation suggests that 200 μmol·m^{-2}·s^{-1} may be sufficient to ensure optimal production for the three species under the tested growing conditions; consequently, an increase by 100 μmol·m^{-2}·s^{-1} does not lead to a rise in production in terms of fresh weight per plant.

Dry matter content was higher in mizuna, whereas the lowest value was recorded in chicory. Light treatments also had a significant effect: T3 showed a 14.2% increase compared to T1 and T2 (6.72 g/100 g FW,

Table 1
Summary of results for all analyzed parameters.

Determination	Species (S)	Light treatments (L)	Interaction S x L
Plant weight	***	ns	ns
Dry matter	***	***	ns
Leaf Area	ns	ns	ns
Folate (5-MTHF)	***	***	ns
NO_3	***	***	***
Al	ns	ns	***
B	***	ns	ns
Fe	ns	ns	ns
Mn	ns	ns	**
Na	***	*	ns
Zn	**	ns	**
P	**	ns	**
Ca	***	***	ns
K	*	ns	ns
Mg	***	ns	**

Data were subjected to two-way ANOVA, and LSD multiple comparison test was used for evaluating the differences among means. ns. not significant. *P < 0.05; ** P < 0.001; *** P < 0.0001.

on average). The increase in DM under constant higher light intensity conditions (300 μmol m^{-2} s^{-1}) is consistent with the greater photosynthetic efficiency induced by elevated PPFD levels, as reported in lettuce (Jin et al., 2023) and other species (Longstreth and Mason 1984). Energy consumption analysis revealed substantial differences among treatments. As reported in the supplementary materials, the total energy input for lighting in T3 was 66% higher than in T1. In contrast, the dynamic strategy T2 resulted in only a 12% increase in energy consumption compared to T1, without increase in folate content.

Folate content, quantified as 5-methyltetrahydrofolate (5-MTHF) — the predominant and most bioavailable folate form in leafy vegetables — showed significant differences both among species and across light treatments. Mizuna and pakchoi exhibited similarly high folate concentrations (156.52 μg/100 g FW, on average) whereas chicory displayed markedly lower levels (Table 2), reflecting the well-documented higher folate density typical of *Brassicaceae* (Farnham et al., 2012; Shohag et al., 2020). This observation aligns with previous findings indicating that species within this family tend to accumulate greater amounts of folate compared with other botanical groups (Zhang et al., 2025). Although varietal differences in folate accumulation have been reported within *Brassicaceae* (Farnham et al., 2012), no significant differences were detected between mizuna and pakchoi in the present study.

Chicory, belonging to the *Asteraceae* family, therefore, appears to have a lower capacity for folate biosynthesis or accumulation under the tested growing conditions.

Light treatments also had a significant effect. The highest folate content was obtained under T3, which provided a constant PPFD of 300 μmol m^{-2} s^{-1} throughout the entire cultivation cycle and resulted in an average increase of 24% compared with the average of T1 and T2. This result clearly indicates a positive influence of increased light intensity on folate accumulation. A possible explanation for the observed increase in folate concentration under higher PPFD is the stimulation of carbon metabolism and the consequent enhancement of precursor availability for folate biosynthesis. Folate synthesis depends on the production of chorismate and guanosine triphosphate (GTP), which originate from primary carbon metabolism, including glycolysis, the tricarboxylic acid cycle and the shikimate pathway. Therefore, higher irradiance may increase photosynthetic carbon assimilation and metabolic fluxes towards these pathways, ultimately promoting folate accumulation. Supporting this hypothesis, Zhu et al. (2025) reported that enhanced carbon flux was associated with increased folate biosynthesis in pakchoi, while Gao et al. (2023) demonstrated that light exposure modulates key enzymes and genes involved in folate metabolism. Although these mechanisms were not investigated in the present study, they may contribute to the higher 5-MTHF concentrations observed under continuous exposure to 300 μmol m^{-2} s^{-1}. However, the influence of light on folate biosynthesis is not unequivocal in literature. The link between folate-mediated one-carbon (1C) metabolism and photorespiration suggests a relationship between light intensity and the biochemical reactions involved in folate synthesis (Okazaki and Yamashita, 2019; Hanson and Roje, 2001). Supporting this hypothesis, recent studies on lettuce demonstrated that plants grown under a higher PPFD (200 μmol m^{-2} s^{-1}) exhibited greater folate concentrations than those cultivated under lower light intensity (150 μmol m^{-2} s^{-1}) (Okazaki and Yamashita, 2019). Additional evidence comes from studies on pea seedlings (*Pisum sativum* L.), which showed that folate concentrations were higher in green leaves than in etiolated seedlings (Gambonnet et al., 2001).

The spectral composition of LED light may also play an important role in regulating folate accumulation. Previous studies have shown that specific wavelengths can influence folate biosynthesis in plants. For example, red LED light (peak wavelength 655 nm) increased total folate concentration in wheat seedlings compared with dark-grown controls (Chang et al., 2021), whereas supplemental red-blue LED combinations enhanced folate accumulation in lamb's lettuce (Długosz-Grochowska et al., 2016). These findings suggest that folate metabolism is influenced not only by light quantity but also by light quality. In the present study, however, all treatments were applied using the same LED spectrum and differed only in PPFD. Therefore, the increase in folate concentration observed under T3 cannot be attributed to any specific wavelength and is more likely associated with the higher light intensity and greater cumulative light exposure. Nevertheless, existing evidence suggests that spectral optimization, particularly through carefully balanced red and blue light combinations, may further promote folate accumulation. Future studies should investigate the interactive effects of PPFD and spectral composition to identify the wavelengths or spectral combinations that most effectively enhance folate biosynthesis, as current evidence suggests that plant responses to light quality are highly species-dependent (Długosz-Grochowska et al., 2016; Voutsinos-Frantzis et al., 2024).

The absence of a significant folate increase under T2 — despite a 50% higher PPFD during the final 7 days — suggests that a sustained

Table 2
Impact of light treatments on plant weight, dry matter, leaf area, folate content, B, Na, Ca and K content in mizuna, pakchoi and chicory baby leaf.

Source of variation	Yield	Dry matter	Leaf area	Folate (5-MTHF)	B	Na	Ca	K
Species (S)	Kg/m^2	g/100 g of FW	mm^2/plant	μg/100 g FW	mg/kg FW			
Mizuna	1.47 b	7.96 a	7711	163.6 a	2.32 a	42 b	3080 a	5987 a
Pakchoi	1.76a	7.10 b	6711	149.4 a	2.56 a	47 b	2866 a	4858 b
Chicory	1.12 c	6.06 c	7477	61.8 b	1.63 b	117 a	919 b	5305 ab
Light treatments (L)								
T1	1.41	6.31b	8028	103.3 b	2.1	64.0 b	2016 b	5785
T2	1.43	7.14 b	6269	128.0 b	2.2	65.5 ab	2438 a	5298
T3	1.52	7.68 a	7602	143.6 a	2.2	76.0 a	2412 a	5067

Data are expressed as mean of the treatment (n = 9). Different letters indicate that mean values are significantly different according to the LSD test (α = 0.05). Cd, Cr, Pb, Mo, and Cu < LOQ. T1: 30 days constant light intensity at 200 μmol/m^2/s; T2: light intensity at 200 μmol/m^2/s until day 23, then increased to 300 μmol/m^2/s; T3: 30 days constant light intensity at 300 μmol/m^2/s.

exposure to elevated light intensity throughout the entire cultivation cycle is required to promote folate accumulation. This is consistent with the light-dependent regulation of HPPK/DHPS expression reported by Jabrin et al. (2003), who observed a gradual increase in enzyme expression during de-etiolation, reaching values comparable to continuous light only after prolonged exposure. Similarly, in maize (*Zea mays* L.), early developmental studies reported a peak in folate concentration under light conditions, which was absent in seedlings germinated in darkness (Liu et al., 2017). Chicory, pak choi, and mizuna were cultivated under varying DLI regimes. In the initial 22 days, T1 and T2 were exposed to 10.08 $mol \cdot m^{-2} \cdot d^{-1}$, whereas T3 received 15.12 $mol \cdot m^{-2} \cdot d^{-1}$. During the last 7 days, the PPFD increase raised the DLI of T2 to 15.12 $mol \cdot m^{-2} \cdot d^{-1}$, which in turn enhanced 5-MTHF content, consistent with findings by Okazaki and Yamashita (2019). Moreover, as reported by Brechner et al. (2007), Xu et al. (2021), Thoma et al. (2020), Min et al., 2026 both DLI and photoperiod can influence multiple metabolic routes, though treatment efficacy may vary considerably across species.

The possibility of increasing folate content using exclusively agronomic protocols had already been demonstrated by Watanabe et al. (2017), who developed a cultivation protocol based on the addition into the nutrient solution of precursors, cofactors, and molecular inhibitors involved in folate biosynthesis, such as phenylalanine, Mg and glutamic acid. However, although effective, this approach requires the use of a silver-based compound to limit bacterial proliferation in the nutrient solution. This aspect implies potential critical issues related both to the disposal of the final product and to the risks associated with producing food using silver salts with antiseptic properties. Other agronomic protocols, such as the one proposed by Bonasia et al. (2025) based on foliar applications of folate synthesis inducers like phenylalanine or salicylic acid, result in only slight increases in folate content.

Regarding mineral composition, B concentrations were higher in pakchoi and mizuna than in chicory, with no significant differences among light treatments. Sodium was markedly higher in chicory than in *Brassicaceae* species, with a slight increase under T3 relative to T1. Calcium content was greater in mizuna and pakchoi (2973 mg kg^{-1} FW, on average), and both T2 and T3 light treatments resulted in a significant 20% increase compared to T1.

Potassium was highest in mizuna, with no significant variation among light treatments (5383 mg kg^{-1} FW, on average). Iron content was not significantly influenced by either species or light treatments and averaged 4.6 mg/kg FW. A significant species × treatment interaction was also observed for several mineral elements, such as Al, Mn, P, Mg and Zn (Tables 1 and 3). Although statistically significant, these interactions were limited to small percentage changes that did not meaningfully affect the nutritional quality of the product, either positively or negatively.

Table 3
Impact of light treatments on accumulation of Al, Mn, P, Mg and Zn in mizuna, pakchoi and chicory baby leaf.

Species	Light treatments	Al mg/kg FW	Mn	P	Mg	Zn
Mizuna	T1	0.84 b	2.52 b	503 c	330 bc	1.96 b
	T2	1.12 ab	2.72 ab	575 bc	406 ab	2.38 ab
	T3	1.21 ab	2.73 ab	599 abc	386 ab	2.43 ab
Pakchoi	T1	0.70 b	2.39 b	631 ab	383 ab	2.26 b
	T2	1.33 ab	2.67 ab	597 abc	387 ab	2.63 ab
	T3	0.74 b	3.60 a	702 a	439 a	2.88 a
Chicory	T1	1.74 a	3.05 ab	646 ab	250 cd	2.66 ab
	T2	0.87 b	2.58 b	594 bc	215 d	1.61 c
	T3	0.62 b	2.31 b	544 bc	162 d	1.36 c

Data are expressed as mean of the treatment (n = 3). Different letters indicate that mean values are significantly different according to the LSD test (α = 0.05). FW: fresh weight. T1: constant light intensity at 200 μmol/m^2/s; T2: light intensity at 200 μmol/m^2/s until day 23, then increased to 300 μmol/m^2/s; T3: constant light intensity at 300 μmol/m^2/s.

However, some species-specific patterns emerged. In pakchoi, both Mg and Zn concentrations reached their highest values under the T3 treatment, contributing to an improved mineral profile of this *Brassicaceae* species (Table 3). Similar species-dependent responses to light intensity have been documented in other horticultural crops, indicating that mineral accumulation is often regulated by both genotype and environmental conditions. In addition, the possibility cannot be excluded that morpho-physiological differences among species, including leaf area and growth rate, may have affected transpiration rates and, consequently, nutrient uptake, thereby contributing to the observed variability. For instance, Chung et al. (2001) observed an increase in Ca concentration in tomato seedlings under higher light intensity, while Grygoray et al. (2015) reported increased K and Ca levels and a reduction in Mg in cucumber (*Cucumis sativus* L.) grown under elevated light intensities.

These findings may be explained by the fact that greater light intensity enhances photosynthetic activity, thereby increasing carbohydrate availability to the roots and promoting active nutrient uptake (Xu et al., 2021). In addition, the differences in mineral element content among species are widely documented in literature. Nutrient uptake is species specific and can also vary according to varieties or populations, as demonstrated in our previous studies (D'Imperio et al., 2016; D'Imperio et al., 2022) and confirmed by other authors (Giordano et al., 2019; Smoleń et al., 2019).

Furthermore, in mizuna, pakchoi, and chicory, the levels of Cd, Cr, Pb, Mo, and Cu were below the limits of quantification (LOQ), underscoring the safety and quality of the products obtained through soilless cultivation systems.

Nitrate accumulation in the three baby leaf species was significantly influenced by both the species and the light treatments applied (Table 1 and Fig. 1). The highest NO_3 values were observed in treatment T1, with no significant differences among the species (average: 4176 mg/kg FW). The lowest values were recorded in pakchoi T3 and mizuna T2 and T3, with an average of 1007 mg/kg FW. In all three species, except for chicory in T2, a statistically significant reduction in NO_3 content was observed under T2 and T3 treatments compared to T1. Moreover, in pakchoi the reduction was correlated with the absolute amount of light received, showing a dose-dependent response. The greatest reduction, 80%, was observed in pakchoi T3 compared to T1 (Fig. 1).

Importantly, the nitrate (NO_3) levels measured in this study were consistently below the maximum limits established by the European Regulation (EC) No. 1258/2011, which sets permitted nitrate concentrations in leafy vegetables such as lettuce and spinach to ensure consumer safety. Similarly, Nájera and Urrestarazu (2019) observed a reduction in nitrate content in vegetables with moderately high nitrate accumulation - including lettuce, rocket, spinach, and endive - when grown under varying light intensities.

This reduction in NO_3 content may be attributed to the stimulatory effect of high light intensity on key enzymes involved in NO_3 assimilation into amino acids, such as glutamine synthetase and glutamate synthase, while simultaneously inhibiting asparagine synthetase, an enzyme involved in the synthesis of non-essential amino acids (Zhang et al., 2018). This enzymatic regulation promotes the incorporation of nitrogen into carbon-rich compounds like glutamine and glutamate. Conversely, under low light conditions, glutamine synthetase and glutamate synthase are suppressed, whereas asparagine synthetase is stimulated. Asparagine, a nitrogen-rich compound, serves to stabilize nitrate for transport or storage, leading to its accumulation in plant tissues.

Fig. 2 (A, B) presents the estimated daily intake and the percentage of the Recommended Dietary Allowance (RDA) for folate provided by a 100 g portion of folate-biofortified baby leaf vegetables for adults (70 kg body weight) and pregnant women. The results show that a standardized 100 g serving (Ninfali et al., 2005) easily incorporated into the daily diet as a salad or side dish, can substantially contribute to meeting the RDA across different population groups. Furthermore, for mizuna and

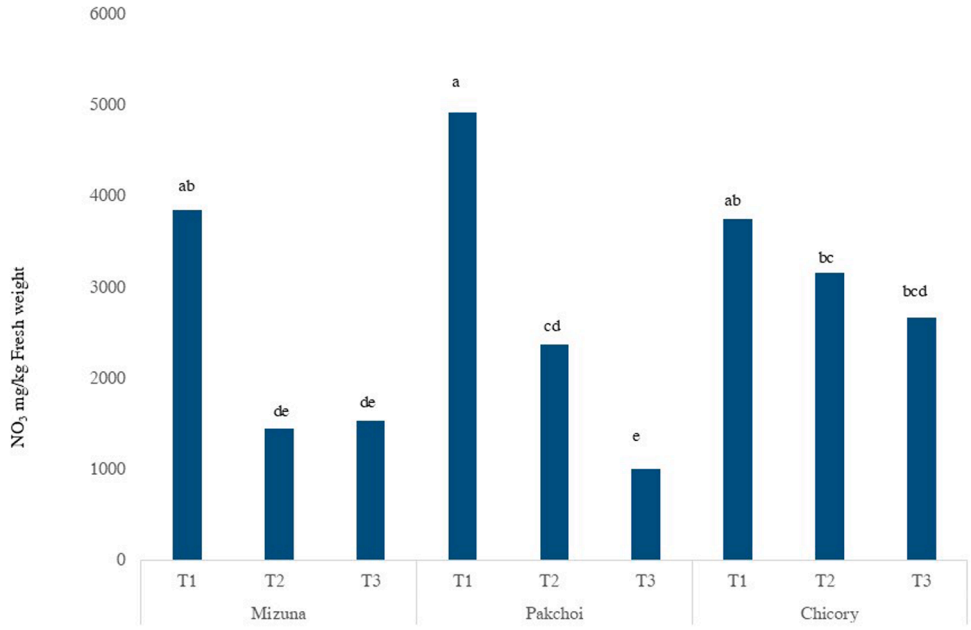

Fig. 1. Impact of light treatments on accumulation of NO_3 in mizuna, pakchoi and chicory baby leaf. Data are expressed as mean of the treatment (n = 3). Different letters indicate that mean values are significantly different according to the LSD test (α = 0.05). (T1: constant light intensity at 200 μmol/m²/s; T2: light intensity at 200 μmol/m²/s until day 23, then increased to 300 μmol/m²/s; T3: constant light intensity at 300 μmol/m²/s).

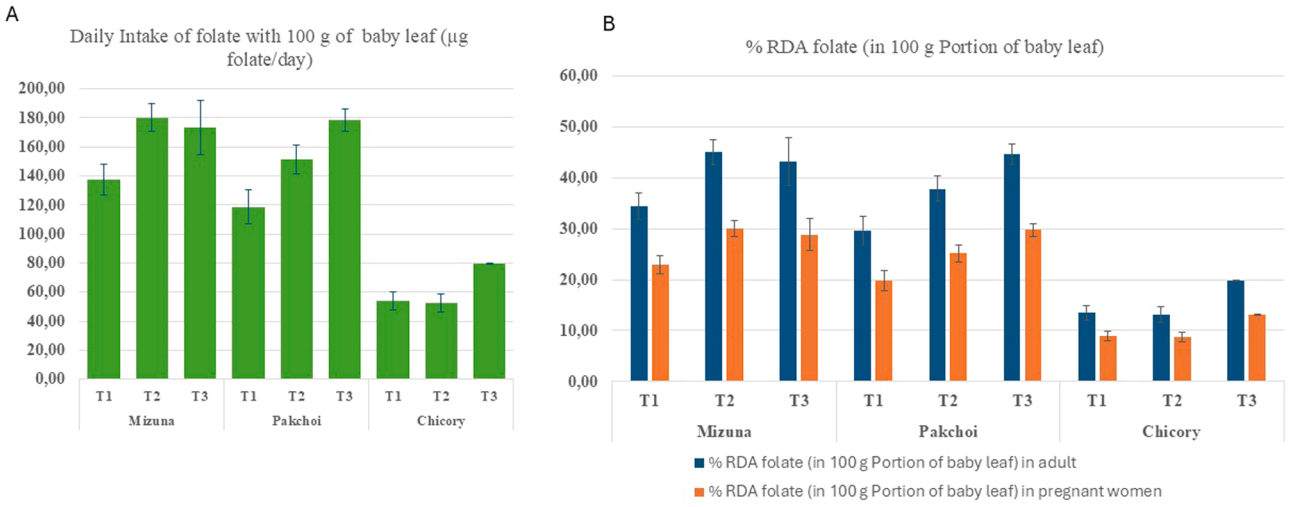

Fig. 2. Estimated daily intake (A), and percentage of RDA (B) for folate derived from the consumption of 100 g portions of folate-biofortified baby leaf vegetables by adults (70 kg body weight) and pregnant women. Data are expressed as mean of the treatment (n = 3). T1: constant light intensity at 200 μmol/m²/s; T2: light intensity at 200 μmol/m²/s until day 23, then increased to 300 μmol/m²/s; T3: constant light intensity at 300 μmol/m²/s.

pakchoi, which can also be consumed cooked, folate levels would be even higher considering a larger portion size of 300 g. Although this aspect needs to be further investigated in relation to the factors affecting folate stability after cooking, previous studies have shown that mild heat treatments, such as steaming, brief boiling, or baking, generally result in higher folate retention compared to more intensive processing (Libenska et al., 2025). For the adult population, whose recommended daily intake is set at 400 μg of folate (Xu et al., 2022), the contribution provided by a 100 g portion varies significantly depending on the plant species considered. Mizuna (T2) and pakchoi (T3) showed the highest folate concentrations, supplying between 180.22 and 178.42 μg per serving, corresponding to approximately 45% of the daily RDA (Fig. 2 B). Therefore, a single meal including one of these portions may cover nearly half of the total daily folate requirement for an adult. Although chicory exhibited lower folate levels (54–79 μg per 100 g), it still provided a meaningful contribution, corresponding to approximately 19% of the RDA. The interest in enhancing folate content in baby-leaf vegetables through light management is strongly supported by the well-documented health benefits associated with adequate folate intake, including the prevention of neurodegenerative disorders, cardiovascular diseases, and neural tube defects during pregnancy (Bekaert et al., 2008). The most critical nutritional window for folate intake is pregnancy, during which the RDA increases to 600 μg day^{-1} to support fetal development and reduce the risk of neural tube defects (Bekaert et al., 2008). In this context, the consumption of 100 g of mizuna (T2) or pakchoi (T3) would provide approximately 30% of the daily requirement.

4. Conclusion

This study demonstrates that light intensity management is an effective and promising strategy to biofortify baby leaf vegetables with folate (5-MTHF) under controlled conditions. The constant high light treatment (T3; 300 μmol m^{-2} s^{-1}) significantly increased folate concentration (+39% on average compared to T1).

Importantly, enhanced PPFD significantly reduced nitrate accumulation, with reductions up to 80% in pakchoi under T3, improving the overall nutritional and safety profile of the product. Mineral composition remained generally stable, and no toxic elements were detected. From a nutritional perspective, a 100 g portion of mizuna or pakchoi grown under T3 can provide up to 45% of the adult RDA for folate, highlighting the practical relevance of this approach. Targeted light management is a promising chemical-free tool for improving micronutrient density and functional quality in baby-leaf vegetables, though success depends heavily on species and genotype selection. To maximize folate content efficiently, future work should assess the combined impact of light intensity, exposure time, spectral composition, and Daily Light Integral (DLI) in order to define the most energy-effective lighting strategies.

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CRediT authorship contribution statement

Massimiliano D'Imperio: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lucia Bonelli:** Writing – review & editing, Writing – original draft, Formal analysis. **Francesco Milano:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Miriana Durante:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Angela Boari:** Writing – review & editing, Formal analysis, Data curation. **Francesco Serio:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization. **Maria Gonnella:** Writing – review & editing, Formal analysis, Data curation. **Angelo Parente:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2026.115134.

Data availability

Data will be made available on request.

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