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LED light distance and split fertilization effects on growth and morphological characteristics of red lettuce in urban cultivation system

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Abstract. Light intensity and nutrients needed for plant growth are important factors in cultivation systems. Information regarding the distance of light sources and fertilization intervals for growing red lettuce in urban cultivation systems is still limited. This study evaluated the effects of LED light distance (35 cm and 50 cm) and split fertilizers (2nd and 3rd) on the growth and morphological of red lettuce in urban cultivation systems. The results showed a significant interaction between light distance and fertilization on morphological traits (leaf, stem, and root growth) and physiological parameters (chlorophyll a, b, and total chlorophyll). The distance of the light source had no significant effect on anthocyanin accumulation. A source light of 35 cm (SL-35) exhibited more dominant vegetative growth in the canopy, as indicated by a higher shoot weight ratio (SWR). The growth was predominantly concentrated in shoot tissues, with maximum leaf size achieved around 14 days after complete leaf expansion. Plants receiving split fertilization reached maximum leaf expansion slightly earlier, at around day 12. The leaf area was accurately estimated using a zero-intercept quadratic linear regression model based on leaf length and width (R^2 = 0.9851).

Key words: adaptation, growth modification, urban agriculture, vegetable diversity.

INTRODUCTION

The limited availability of agricultural land in urban areas, due to land conversion for residential and industrial use, has resulted in fewer cultivation activities (Azadi et al., 2021). Additionally, increasingly dense and tall buildings obstruct and limit sunlight exposure, which consequently affects plant morphology. Various types of cultivation systems can be implemented in urban areas. One potential solution is indoor farming, which enables plant cultivation without reliance on large land areas and is less affected by climatic fluctuations.

The variety of vegetables commonly cultivated in urban areas is relatively limited, resulting in a limited diversity of locally grown produce. One type of vegetable with the potential for development that is widely favoured is lettuce. There are various types of lettuce with different morphologies, including leaf shape and color. Lettuce (*Lactuca sativa* var. crispa), which has high economic value, is one type of lettuce that can be developed to increase the diversity of vegetables in urban areas. However, the conventional cultivation of red lettuce in urban areas remains limited, often due to restricted growing space and the risk that climate change will affect its morphological characteristics. In addition, direct light exposure conditions can increase the risk of plant damage (Shi et al., 2022).

In indoor cultivation systems, artificial lighting and shading is a crucial factor influencing plant growth and physiology. Shading has direct and indirect effects on plant growth and development since shading alters microclimate condition underneath the shading (Fadilah et al., 2022). Using Light-Emitting Diode (LED) lamps is widely used because of its energy efficiency and ability to provide adjustable light spectra suited to plant needs (Barcelo et al., 2021). Modifying the spectrum and intensity of LED light is crucial for optimal photosynthesis and accelerated growth red lettuce. The red (around 660 nm) and blue (around 450 nm) spectra of LED lights have been shown to significantly promote leaf growth, biomass formation, and anthocyanin pigment accumulation in red lettuce (Boros et al., 2023). However, light intensity also depends on the distance between the source and the plant canopy. The distances that are too far apart can reduce reduce photosynthetic efficiency, whereas too close a distance may cause excessive heat and energy use (Zhang et al., 2022a). Optimizing this distance is therefore crucial for efficient indoor cultivation.

Nutrient management, particularly fertilization, is a crucial factor in indoor cultivation systems alongside lighting. The split fertilization strategy, which involves providing fertilizer in stages according to the stage of plant growth, is thought to enhance nutrient absorption efficiency and promote optimal morphological development and yields (Priya et al., 2024). In addition, De Aquino (2021) suggests that split fertilization of phosphorus (P) fertilizer is a strategic approach to prevent fixation, thereby maintaining nutrient availability throughout the crop cycle.

Although various studies have been conducted on the effect of LED light on increasing plant growth and yield (Rahman et al., 2021), most have focused primarily on the influence of light spectrum or intensity (Cammarisano et al., 2021; Alrajhi et al., 2023; Qiao et al., 2025). However, the research about exploring the combined effects of LED light distance and split fertilization, particularly in indoor red lettuce cultivation under controlled conditions is still limited. Most of these studies have focused exclusively on testing the impact of different LED light spectrums (Cammarisano et al., 2021; Alrajhi et al., 2023; Qiao et al., 2025), without considering other cultivation factors such as split fertilization strategies. The research focusing on the combination of treatments involving purple LED lights and plants, as well as split fertilization, to enhance the morphological growth, quality, and yield of red lettuce cultivated indoors in urban environments has not been conducted extensively. Therefore, this study provides new insight into how these two factors interact to optimize growth, yield, and physiological traits of red lettuce in an urban indoor system.

MATERIALS AND METHODS

Research site

The research was conducted at an outdoor agricultural research facility at Jakabaring (104°46'44" E, 3°01'35' S), Palembang, South Sumatra, Indonesia. The study commenced on August and concluded on September, 2024. The research is situated in a tropical lowland.

Research preparation and cultivation procedure

A 2-inch diameter PVC pipe frame is used to construct a dark ‘cube-house’ measuring 4 meters in length, 2 meters in width, and 4 meters in height. All sides of the cube house are lined with plywood, except for the floor were lined with plywood and wrapped in thick black plastic to prevent sunlight penetration and rainwater leakage. The cube is installed with air vents made from 14-inch-diameter PVC pipes, measuring 6 metres in length. Thus, this large-diameter pipe is positioned to penetrate both sides of the cube, protruding one meter out. Small holes along the vent pipes allowed hot air to escape.

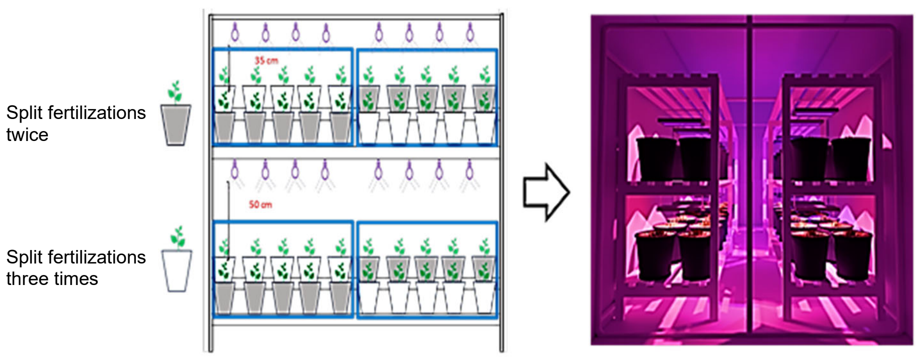

Figure 1. The illustration of research side.

Inside, two shelves (left and right) with two tiers each (top and bottom) were installed for pot placement, measuring 3.75 m × 0.8 m × 1.8 m (l × w × h) (Fig. 1). Pots measured 27.5 cm in height and diameter, filled with a 3:1 (v/v) mixture of mineral soil and cow manure. Soil analysis was conducted, which included measurements of pH H_2O of 5.7, total nitrogen (N) content of 0.11%, available phosphorus (P) of 111 mg kg^{-1}, available potassium (K) of 142 mg kg^{-1}, organic carbon (C-organic) content of 3.64%, and a soil texture consisting of 57% sand, 9% silt, and 34% clay. Seedlings were transplanted at 14 days old. Fertilizer (NPK 16:16:16) was applied 7 days after transplanting. The time for fertilizer application consists of two applications, each with a dose of 1.5 g per pot, for the second and third split fertilization, with a dose of 1 g per pot for each application.

The light source within the cube was supplied by 20 equally spaced 50-watt purple LED (Light-Emitting Diode) bulbs for the 32 m^3 of space within the cube. The LED used was of the SMD2835 type, emitting purple light with wavelength ranging from 395 nm – 420 nm. The light was turned on for 16 hours per day. At the 35 cm distance, the estimated light intensity was approximately 185 μmol m^{-2} s^{-1}, while at 50 cm it was approximately 111 μmol m^{-2} s^{-1}.

Data collection

The data collected consisted of the growth and yield parameters of red lettuce, as well as microclimate conditions. Plant growth data were collected starting from 1 week after planting (WAP), including the daily leaf growth rate, leaf length, and leaf width. Measurement of the daily leaf growth rate began when the leaves were fully opened and continued until leaf growth reached its optimum.

Weekly vegetative growth data included the number of leaves and the canopy area. The canopy area was measured using digital scanning with the Easy Leaf Area application for Android. Yield data were collected at 4 WAP and included the fresh and dry weights of leaves, stems, roots, and total biomass. In addition, stem length and diameter, root length and width, and leaf thickness were recorded, and leaf thickness was measured using a digital caliper.

Furthermore, the root weight ratio (RWR), shoot weight ratio (SWR), and shoot root ratio (SRR) were also calculated. Weight-related variables were measured using a digital scale. A leaf area (LA) estimation model was developed based on directly measured leaf length and width data. Direct measurements of LA were obtained using LIA32 digital image analysis software developed by Kazukiyo Yamamoto (Nagoya University, Japan). The LIA32 software is capable of recognizing the full visible color spectrum (Kitao et al., 2022). Leaf length (L) and width (W) were used as predictors, either individually or in combination (LW). A zero-intercept linear model was used when LW was applied as the predictor.

Measurements of chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin, and carotene were also conducted. Meanwhile, microclimate data included air temperature, humidity, and light intensity. Temperature and humidity were measured using a Tzone TempU07B data logger. Light intensity was measured using a Benetech GM1030 lux meter.

Estimation of Chlorophyll Content

The 1 g of fresh tissue from each leaf was extracted in 100 mL of 80% (v/v) acetone. The absorbance of the extracting solution at 646 nm and 663 nm was measured by a spectrophotometer (UV-3150, Shimadzu Corporation, Kyoto, Japan). The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the equation (Sims & Gamon, 2002).

Research design and statistical analysis

The study was conducted using a factorial randomized complete block design (RCBD). The first factor was the distance of the light source from the plant, consisting of two levels: 35 cm (SL-35) and 50 cm (SL-50). The second factor was the frequency of split fertilization, composed of two applications (2^{nd}) and three applications (3^{rd}). Each treatment combination was replicated five times.

All collected data were analyzed using analysis of variance (ANOVA), and treatment means were compared using the least significant difference (*LSD*) test at a significance level of $P < 0.05$. Additionally, selected parameters were analyzed using simple regression to determine their relationships with each other. All statistical analyses were performed using RStudio (version 2023.06.0+421) on the Windows 10 operating system. The results were presented in the form of tables and figures.

RESULTS AND DISCUSSION

Morphology and physiology of red lettuce

The morphological growth of leaves, stems, and roots serves as an indicator of plant adaptation and tolerance to specific environmental conditions. The analysis results indicated that the distance of the light source had a significant effect on the morphological growth of red lettuce. The 35 cm light distance (SL-35) stimulated faster development of leaves, stems, and roots. In contrast, the split fertilization treatment had no significant effect on leaf and root growth. The combination treatment of SL-35 with three fertilizations (3^{rd}) resulted in enhanced overall growth, as evidenced by improvements in the morphological traits of red lettuce (Table 1).

Table 1. The effect of source light distance and split fertilization on morphological of red lettuce

Treatment	Leaf			Stem		Root	
	Length (cm)	Width (cm)	Length (mm)	Length (cm)	Diameter (mm)	Length (cm)	Width (cm)
Source light							
SL-35	10.15 a	8.70 a	0.52 a	12.83 a	5.94 a	15.12 a	7.80 a
SL-50	8.55 b	6.59 b	0.19 b	9.98 b	4.24 b	11.75 a	3.65 b
LSD	0.75	0.58	0.14	1.52	0.63	3.38	1.39
Split fertilization							
2^{nd}	9.27 a	7.71 a	0.31 a	11.78 a	4.65 b	13.62 a	5.20 a
3^{rd}	9.27 a	7.58 a	0.41 a	11.03 a	5.53 a	15.12 a	6.25 a
LSD	0.75	0.58	0.14	1.52	0.63	3.38	1.39
Interaction of SL and SF							
SL-35:3^{rd}	10.41a	8.83 a	0.61 a	13.22 a	6.79 a	15.87 a	8.75 a
SL-35:2^{nd}	9.90 a	8.58 a	0.44 a	12.45 ab	5.09 b	14.37 ab	6.85 a
SL-50:3^{rd}	8.64 b	6.85 b	0.21 b	10.35 bc	4.28 b	12.87 ab	3.37 b
SL-50:2^{nd}	8.47 b	6.34 b	0.18 b	9.62 c	4.21 b	10.62 b	3.55 b
LSD	1.07	0.82	0.21	2.15	0.89	4.79	1.97

Note: Means followed with the same letters within columns are not significantly different based on the *LSD* at $p \leq 0.05$; ns: not significantly different. SL-35 = source light at 35 cm, SL-50 at 50 cm, 2^{nd} = twice fertilization period, 3^{rd}= three times fertilization period. *LSD* = least significant difference.

The morphological growth of plants, including the development of leaves, stems, and roots, is a crucial indicator of the activation of plant adaptation and physiological processes in response to specific environmental conditions. Lettuce plants have shown significant morphological growth. However, red lettuce cannot grow optimally under LED lighting in terms of physiological growth. The classical reaction of plant adaptation in response to changes in light conditions is characterized by change in plant growth (Korsakova et al., 2019). The SL-35 LED light source has been demonstrated to stimulate the growth of leaves, stems, and roots. This indicates that the intensity and distribution of light from an SL-35 LED light source optimally promote photosynthesis and metabolic processes in plants, thereby encouraging the growth of morphological characteristics of a higher quality (Zakurin et al., 2020). Red lettuce growth is more dominant in the shoot than in the roots. This is because the leaves are more responsive to light as an adaptive response to optimise light absorption for growth. The distance of the light source influences more active leaf growth. Leaf area and thickness are influenced by the efficiency of photosynthesis, where sufficient light enables plants to increase carbohydrate production and strengthen the tissue structure (Dai et al., 2024). The morphological characteristics observed in this study were significantly influenced by the distance of the LED light source, with increased distances amplifying the effects. The closer distance of the LED light source tends to stimulate plant growth, which affects the morphological characteristics of red lettuce plants. However, lettuce plants can respond differently depending on the type. Reeza et al. (2024) also found that different plants have different light requirements. The differences in response between lettuce varieties provide an opportunity to select those suited to indoor cultivation conditions, thereby increasing the diversity of vegetables available in urban areas.

Using an LED light source at a distance of around 35 cm (SL-35) still enables the production of vegetables of good morphological quality. According to Miao et al. (2023), the morphological response of lettuce plants cultivated with artificial light at an intensity of 300 $\mu mol\ m^{-2}\ s^{-1}$ differs between varieties. They recommend adjusting the light intensity specifically to the type and variety of plant. Jeong et al. (2024) found that the light spectrum and air temperature interactively regulate the morphology and subsequent growth of lettuce plants. Therefore, it should be adjusted together within a controlled environmental system. In addition to changing the distance of the LED light source, the nutritional needs of plants also influence their morphological and physiological characteristics. Fertilizer application and timing, tailored to the plant's phase and needs, will support the growth process. The availability and proportion of nutrients during the growth phase directly affect morphological characteristics (Hong et al., 2022). The results of this study show that third split fertilization directly increases the morphological attributes of red lettuce, including leaf, stem and root size. It is thought that this is because the nutrients required by lettuce during its growth phase become available gradually from the beginning of this phase until the optimal stage before harvest. As plant growth and development are primarily determined by nutrient availability, it is essential to consider the dynamics of nutrient absorption and transport in plants (Kumar et al., 2021).

The physiological and morphological development of red lettuce grown indoors was influenced by both the distance of the LED light source and the frequency of split fertilization. In addition to physiological responses, changes in leaf morphology were also observed. Plants grown under the SL-35 light distance exhibited denser foliage, longer, and more branched roots, suggesting more active root development. Conversely, red lettuce cultivated under the SL-50 light source had a sparser canopy and shorter, less developed roots (Fig. 2). Morphological diversity is not only found in the leaves but also the root system. The increased growth of roots in SL-35 is attributed to the enhanced distribution of photosynthates to the roots in response to more intense and efficient lighting (Balliu et al., 2021). A closer light distance increases chloroplast activity and photosynthetic carbon assimilation. The higher production of assimilates that are not only effected for leaf expansion but also translocated to the roots to support elongation and nutrient uptake (Liu et al., 2023). The optimal growth in canopy and root that grown under SL-35 may therefore indicate a balanced source sink by optimal light intensity.

The SL-35 treatment also induced physiological changes, particularly in the accumulation of anthocyanin pigments. A low anthocyanin concentration resulted in predominantly green leaves with only a slight reddish hue, which is typical for red lettuce (Fig. 3). The physiological process of red lettuce leaves has a significant influence on its visual appearance, particularly in terms of leaf color. This may occur because the red coloration in lettuce leaves, which is mainly caused by the biosynthesis and accumulation of anthocyanin pigments, is highly influenced by light intensity. The red coloration of lettuce leaves is primarily driven by the physiological accumulation of anthocyanin pigments, which respond to environmental factors such as light intensity (Wada et al., 2022). These findings are consistent with previous studies indicating that controlled LED lighting environments can be adjusted to promote plant growth, pigment accumulation, and nutraceutical quality in leafy vegetables (Rahman et al., 2021). The pigments not only enhance the visual appeal of the plant but also reflect its adaptive responses to indoor growing conditions.

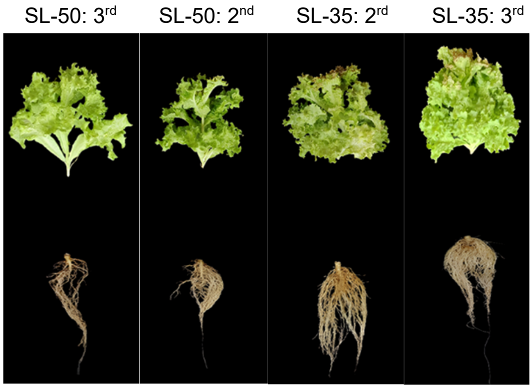

Figure 2. Visualization of morphology shoot and root of red lettuce under different source light distance and time split fertilizer.

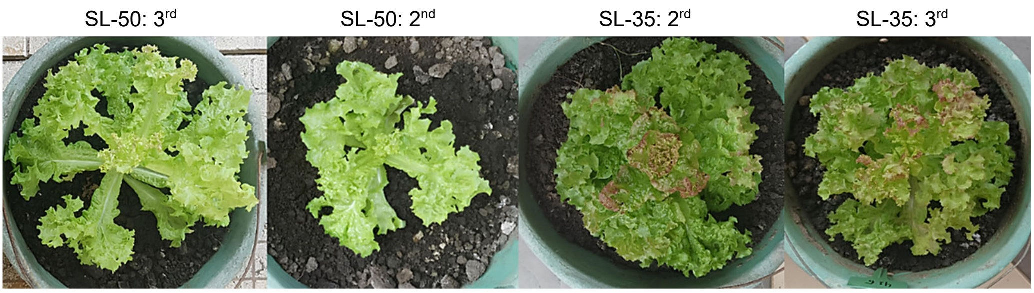

Figure 3. Visualization of canopy area of red lettuce under different source light distance and time split fertilizer.

The results of the study indicated that pigment accumulation in red lettuce leaves was influenced by both the distance of the LED light source and the split fertilization. Application of LED lighting at a closer distance of 35 cm (SL-35) statistically significantly increased the content of chlorophyll a and total chlorophyll compared to lighting at 50 cm (SL-50). Although the differences in chlorophyll b, anthocyanin and carotenoid content were not statistically significant, there was a tendency for higher concentrations of these functional pigments under the SL-35. In addition, split fertilization statistically significant on total chlorophyll. The three-time split fertilization (3rd) resulted in significantly higher chlorophyll a and total chlorophyll content than the two-time split fertilization (2nd). This suggests that fertilization carried out gradually during the growth phase can support more optimal green pigment synthesis, the crucial process for plant growth and development. The interaction between the distance of the light source and split fertilization had a highly significant effect. The combined treatment of SL-35 LED lighting and three fertilization applications (SL-35:3rd) resulted in the highest levels of all measured pigment parameters. In contrast, the SL-50:2nd combination resulted in the lowest pigment accumulation, including significant reductions in chlorophyll a and total chlorophyll content. Overall, these findings suggest that closer LED light placement combined with gradual fertilization enhances the accumulation of both photosynthetic pigments (chlorophylls and carotenoids) and antioxidant pigments (anthocyanins) in red lettuce grown under indoor cultivation conditions (Table 2).

Table 2. Comparison of pigment quantity and ratio between Chlorophyll– A, Chlorophyll– B, Anthocyanin, Carotenoids and Total Chlorophyll of red lettuce under different source light distance and time split fertilizer

Treatment	Chl – A (mg g^{-1})	Chl – B (mg g^{-1})	Anthocyanin (mg g^{-1})	Carotenoids (mg g^{-1})	Total Chl (mg g^{-1})
Source light					
SL-35	1.703 a	0.468 a	0.159 a	0.250 a	2.171 a
SL-50	1.277 b	0.378 a	0.075 a	0.403 a	1.655 b
LSD	0.271	0.130	0.135	0.195	0.354
Split fertilizer					
2nd	1.260 a	0.341 a	0.131 a	0.311 a	1.602 b
3rd	1.719 a	0.505 a	0.102 a	0.342 a	2.171 a
LSD	0.271	0.130	0.135	0.195	0.354
Interaction of SL and SF					
SL-35:3rd	1.934 a	0.516 a	0.201 a	0.404 a	2.451 a
SL-35:2nd	1.504 b	0.494 a	0.116 a	0.401 a	1.998 ab
SL-50:3rd	1.472 b	0.419 ab	0.089 a	0.280 a	1.892 b
SL-50:2nd	1.049 c	0.263 b	0.060 a	0.221 a	1.313 c
LSD	0.383	0.184	0.191	0.275	0.501

Note: Means followed with the same letters within columns are not significantly different based on the *LSD* at $p \leq 0.05$; ns: not significantly different. SL-35 = souce light at 35 cm, SL-50 at 50 cm, 2nd = twice fertilization period, 3rd= three times fertilization period. *LSD* = least significant difference.

The leaf is a crucial organ for the process of photosynthesis, which involves the use of light intensity. Visually, red lettuce leaves have not shown optimal red pigment formation. The red color on the leaves appears uneven, indicating a low level of anthocyanin pigment synthesis. The duration of exposure and the distance of the LED light source are not optimal, which has affected the environmental conditions, making them less supportive of the physiological processes of red lettuce. The ability of plants to produce pigments, particularly anthocyanins in leaves, is significantly influenced by environmental growing conditions, including the intensity and quality of light (Anum et al., 2024). The concentration of anthocyanins increases when plants are exposed to light of specific wavelengths, particularly blue and red light, which stimulates the biosynthesis of pigments (Modarelli et al., 2022).

In addition to influencing leaf morphology, anthocyanins are involved in processes such as growth and yield (Palsha et al., 2024). The light intensity received by the plant is affected by the distance of the LED light source to the plant. The distance of the LED light source statistically significant on chlorophyll a and total chlorophyll. Furthermore, the closer light source distance of 35 cm (SL-35) indirectly increases the light intensity received by the plants. In this study, the increase in chlorophyll content occurred due to the closer distance to the LED light source (SL-35). This suggests that higher light intensity, resulting from the distance of the light source, can enhance pigment synthesis in red lettuce leaves, thereby increasing chlorophyll accumulation (Kong & Nemali, 2021). Generally, plants respond to high light intensity by increasing their pigment content to maximize light energy absorption (Liu et al., 2021). However, the light source is too far away, the distance reduces the accumulation of chlorophyll because the light intensity received by the plant is insufficient to support optimal photosynthesis (Wang et al., 2022). Light of a specific wavelength can stimulate the enzymes involved in the synthesis of chlorophyll and anthocyanin (Soufi et al., 2023b).

Meanwhile, split fertilization statistically significant on total chlorophyll. The 3rd split fertilization tends to produce optimal total chlorophyll accumulation. Applying fertilizer gradually based on the plant's growth phase encourages pigment biosynthesis in the leaves. The optimal chlorophyll accumulation is likely related to sustained nitrogen availability throughout the growth phase. Nitrogen is important in chlorophyll biosynthesis as a structural element (Zhou et al., 2021). The gradual fertilizer application ensures continuous nutrient supply for pigment synthesis and prevents nutrient stress that could limit chlorophyll formation (Ojeniyi et al., 2024). Gradual and sustained application of fertilizer allows plants to absorb nutrients at the right time according to growth phase, especially during the active phase of photosynthesis and leaf pigment biosynthesis.

The interaction between the light source and split fertilization increases the red pigment content of lettuce. Therefore, adjusting the intensity and quality of light in conjunction with optimal fertilization time is a factor in plant physiological responses, including photosynthetic efficiency and leaf visual quality, such as color and intensity. This finding indicates a synergistic relationship between light needed for photosynthesis and nutrient availability. Source light at 35 cm distance, higher light intensity enhanced photosynthetic activity and metabolic processes, while gradual nutrient supply maintained continuous availability of essential elements, promoting the synthesis of chlorophyll and stable pigment (Sharma et al., 2025). According to Soufi et al. (2023a) balanced light and nutrient conditions improved photosynthetic efficiency and biomass accumulation, demonstrating the synergistic influence of light intensity and nutrient supply in enhancing photosynthetic performance to enhance growth of red lettuce in controlled environments.

The growth and yield of red lettuce

The results showed that the SL-35 LED light source, positioned at a closer distance, was more effective in promoting an increase in leaf number and supporting optimal vegetative growth. Maximum leaf development began to occur in the third week after transplanting, indicating that no significant differences were observed between treatments during the early growth phase. In contrast, split fertilization did not significantly affect leaf growth in red lettuce. However, a slight trend of increased leaf number was observed under the three-time split fertilization (3rd) treatment (Fig. 4).

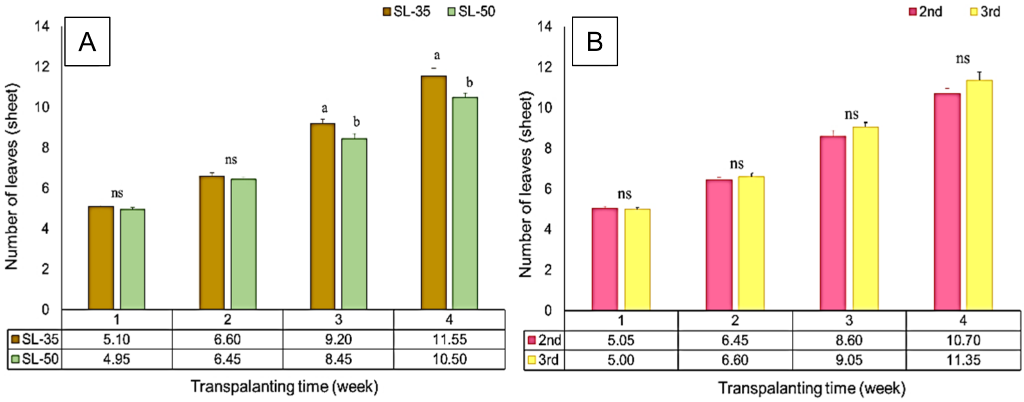

Figure 4. The effect of source light distance and split fertilization on number of leaves. Means followed with the same letters within columns are not significantly different based on the *LSD* at $p \leq 0.05$; ns: not significantly different. SL-35 = souce light at 35 cm, SL-50 at 50 cm, 2^{nd} = twice fertilization period, 3^{rd} = three times fertilization period.

The leaves of red lettuce plants are commercially valuable. The growth during the vegetative phase typically begins with leaf development, characterized by an increase in the number of leaves. The initiation of new leaf growth in red lettuce was stimulated by a closer LED light source distance of 35 cm (SL-35). This may be due to the relatively low nutrient demand of red lettuce during the early vegetative stage, where initial fertilization were sufficient to support leaf growth (Hong et al., 2022). Leaf development during this phase is mainly driven by light availability rather than nutrient supply, and in this study, a closer LED light distance of 35 cm (SL-35) effectively stimulated new leaf initiation. The response to split fertilization tends to appear in biomass accumulation during later vegetative stages, when photosynthesis and nutrient uptake increase (Zhou et al., 2019). Therefore, adjusting the timing of fertilizer application according to the plant's growth stage may improve nutrient uptake efficiency and enhance overall growth performance (Li et al., 2023).

The distance of the LED light source had a significant effect on vegetative growth, particularly on canopy area expansion. In this study, the growth of the canopy area was significantly affected by the distance of the LED light source, which was 35 cm (SL-35). Meanwhile, the growth of the red lettuce plant canopy did not show any significant differences due to the 2nd and 3rd split fertilization, although there was a tendency for an increase in the 3rd split fertilization (Fig. 5). The expansion of the canopy area correlates with the initiation of new leaves. The growth and initiation of red lettuce leaves are influenced by environmental factors, such as lighting and nutrient availability (Zhou et al., 2019). Light is the primary energy source for photosynthesis. The optimal distance for supporting the growth of red lettuce leaves is 35 cm (SL-35), where leaf initiation is significantly different when the distance is greater than this. Additionally, third split fertilization affected the growth of new leaves in red lettuce plants. Similar effects were also observed in canopy growth. Similar trends were also observed in the growth of the canopy area. Gradual fertilization was effective in providing a sustained supply of nutrients for the plants. Nutrients supplied through fertilization play a crucial role in maintaining the metabolic balance of plants (Tei et al., 2020). This has a positive effect on plant growth, particularly during the vegetative growth phase.

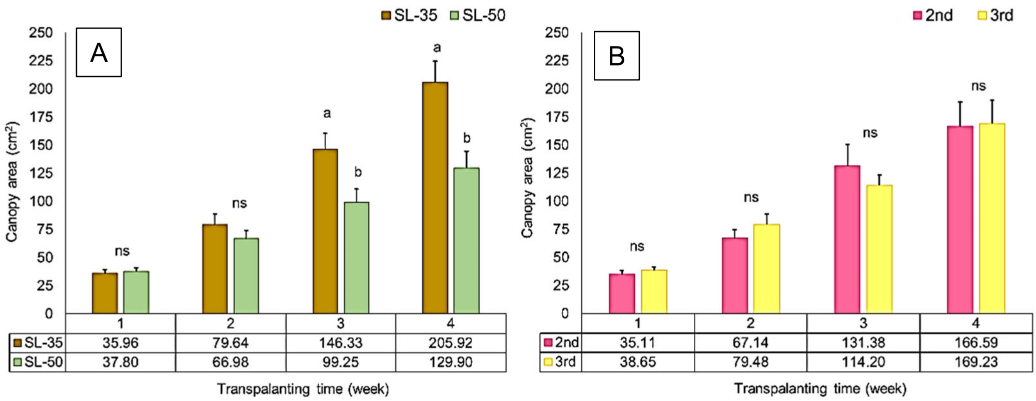

Figure 5. The effect of source light distance and split fertilization on canopy area. Means followed with the same letters within columns are not significantly different based on the *LSD* at $p \leq 0.05$; ns: not significantly different. SL-35 = souce light at 35 cm, SL-50 at 50 cm, 2^{nd} = twice fertilization period, 3^{rd} = three times fertilization period.

The measurements of leaf growth rate show that leaf length and width change over 14 days after the leaves unfold. The daily leaf growth rate due to LED light source distance treatment tends to be longer compared to split fertilization. The results of this study show that the growth of leaf length and width due to differences in the distance of the light source is longer and begins to reach maximum size from day 13 to 14 after the leaves unfoolded. Meanwhile, leaf growth resulting from split fertilization tends to be shorter, reaching its maximum size by day 11. Additionally, leaf growth increased rapidly at the beginning of the period, slowing down on day 10, indicating that the leaves had reached their maximum size. Growth in leaf width appeared slightly slower than growth in leaf length, but continued until day 13 (Fig. 6). Red lettuce leaf growth occurs over approximately 14 days. The distance of the LED light source affects leaf growth dynamics, including the expansion of leaf blades. Growth in leaf width tends to cease earlier than growth in leaf length. Furthermore, the number of leaves, the growth rate of leaves, and the size of leaf blades are also affected by the distance between the *LED* light source and the red lettuce plants. Meanwhile, split fertilization affects both leaf length and width. The growth of these traits tends to cease earlier due to the gradual distribution of nutrients throughout the plant. The application of fertilization in stages can influence nutrient distribution and growth balance, leading to an earlier cessation of leaf development (Li et al., 2023).

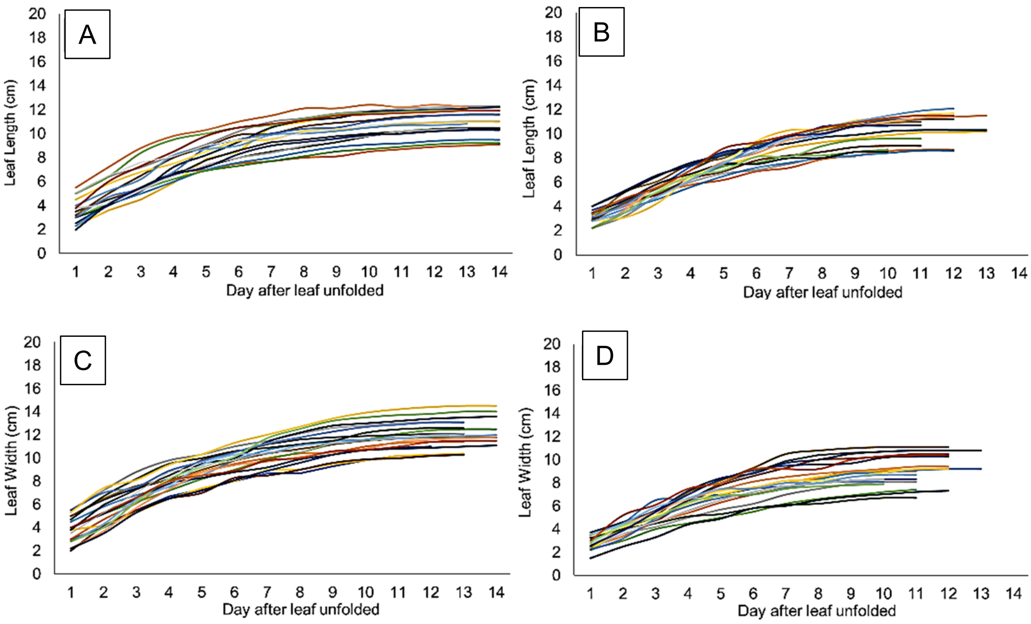

Figure 6. The leaf growth rate based on length and width in red lettuce affect source light distance SL-35 (A, C) and split fertilization (B, D).

The increase in leaf growth under the SL-35 treatment contributed to a more dominant canopy structure. Growth was more concentrated in the shoot system than in the root system, as reflected by the higher shoot weight ratio (SWR) and shoot-to-root ratio (SRR). Overall, the results indicate that while the LED light distance and split fertilization affected shoot development, these treatments did not significantly alter the allocation of biomass between the shoot and root systems (Fig. 7). The observed increase in leaf growth under the SL-35 treatment suggests a favorable response of the shoot system to closer LED light proximity. This treatment promoted a more prominent canopy development, which is consistent with the elevated values of shoot weight ratio (SWR) and shoot-to-root ratio (SRR), indicating a preferential allocation of resources to shoot growth. This condition likely improved chlorophyll synthesis and carbohydrate accumulation, thereby promoting shoot expansion. These findings align with studies reporting that light intensity plays a pivotal role in modulating shoot expansion and leaf biomass accumulation (Eghbal et al., 2024). Similar finding also reported by Esmaili et al. (2020**)** who demonstrated that increasing light intensity within an optimal range can stimulate photosynthetic efficiency and promote shoot development in lettuce. However, despite variations in shoot development and the overall biomass partitioning between shoot and root systems, there was no statistical difference between treatments.

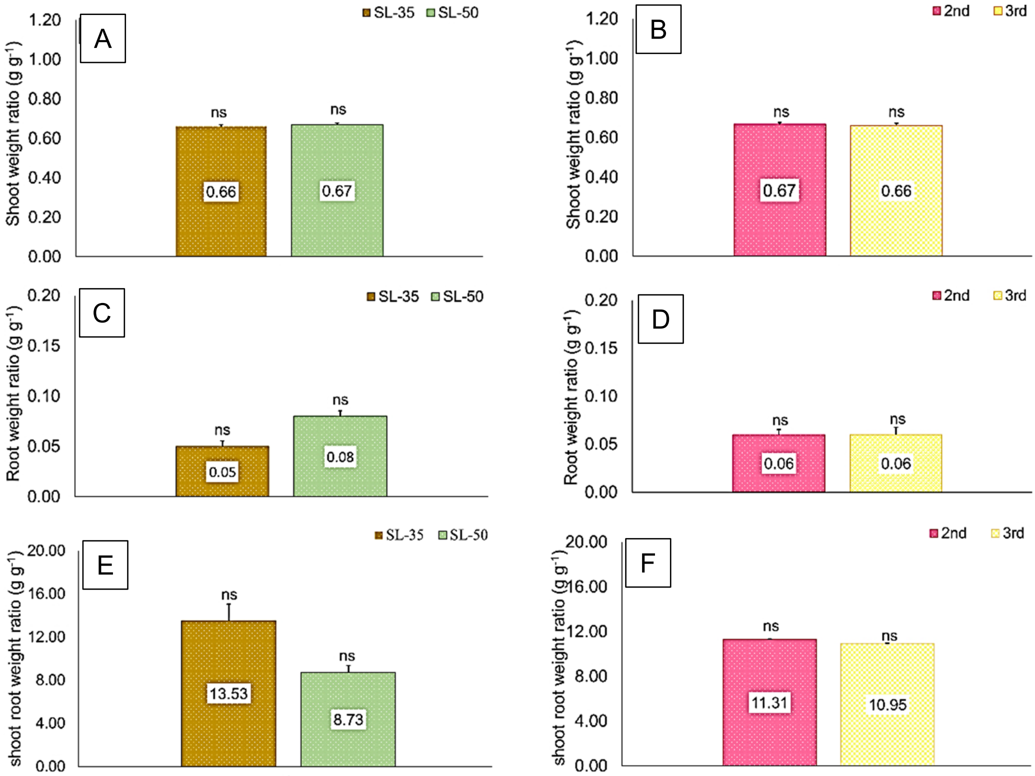

Figure 7. Shoot weight ratio (SWR) (A-B), root weight ratio (RWR) (C-D), (B) and shoot-root ratio (SRR) (E-F) of red lettuce under different source light distance and time split fertilizer. Means followed with the same letters within columns are not significantly different based on the *LSD* at $p \leq 0.05$; ns: not significantly different. SL-35 = souce light at 35 cm, SL-50 at 50 cm, 2nd = twice fertilization period, 3rd = three times fertilization period.

The main part of red lettuce that is consumed is the leaf blade. The growth of lettuce is affected by the distance of the LED light source. The distance has a significant effect on the accumulation of plant biomass. The distance of the SL-35 LED light source shows a substantial increase in fresh and dry biomass for all plant components (leaves, stems, roots, and total biomass). Additionally, there is a tendency that the further the distance between the LED light source and the object, the greater the effect on the object. The plant biomass yield is decreased by the LED light source (50 cm). In addition, the 3rd split fertilization yielded higher results compared to the 2nd split fertilization, particularly in leaf dry weight and total biomass. The interaction between shade and split fertilization indicated that the most effective combination was the SL-35 treatment with split fertilization SL-35:3rd, which produced the highest total fresh and dry biomass. This suggests that the distance between the SL-35 LED light source and the split fertilization 3rd can significantly enhance plant growth and biomass accumulation (Table 3).

Table 3. The effect source light distance and split fertilization on fresh and dry weight red lettuce

Treatment	Leaves		Stem		Root		Biomass	
	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
Source light								
SL-35	29.59 a	1.42 a	3.62 a	0.21 a	3.10 a	0.20 a	36.32 a	2.44 a
SL-50	11.65 b	0.60 b	1.41 b	0.08 b	0.78 b	0.05 b	13.84 b	1.05 b
LSD	4.59	0.33	1.01	0.08	1.13	0.08	6.52	0.60
Split fertilizer								
2nd	16.45 a	0.79 b	2.11 a	0.12 a	1.41 a	0.09 a	20.31 b	1.37 b
3rd	24.45 b	1.23 a	2.91 a	0.17 a	2.47 a	0.16 a	29.85 a	2.11 a
LSD	4.59	0.33	1.01	0.08	1.13	0.08	6.52	0.60
Interaction of SL and SF								
SL-35:3rd	37.16 a	1.78 a	4.46 a	0.27 a	4.18 a	0.27 a	45.81 a	3.02 a
SL-35:2nd	22.01 b	1.06 b	2.78 b	0.15 b	2.03 b	0.12 b	26.82 b	1.86 b
SL-50:3rd	11.74 c	0.68 bc	1.45 b	0.08 b	0.80 b	0.05 b	13.88 c	1.20 bc
SL-50:2nd	11.54 c	0.52 c	1.37 b	0.07 b	0.77 b	0.05 b	13.80 c	0.89 c
LSD	6.49	0.47	1.43	0.11	1.60	0.11	9.22	0.85

Note: Means followed with the same letters within columns are not significantly different based on the *LSD* at $p \leq 0.05$; ns: not significantly different. SL-35 = source light at 35 cm, SL-50 at 50 cm, 2nd = twice fertilization period, 3rd = three times fertilization period. FW = Fresh Weight, DW = Dry Weight. *LSD* = least significant difference.

Morphological characteristics of plants, including leaves, stems, and roots, are directly related to biomass accumulation, as these organs play a critical role in determining both fresh and dry weight accumulation. Leaves are the main component in the cultivation process and biomass accumulation. The proximity of the light source to the plant affects leaf growth. In indoor cultivation systems, the distance between the light source and the plant canopy has a direct influence on biomass accumulation. Light is the energy source that plants primarily use to support the photosynthesis process. An excessive distance between the LED light source and the plants reduces the light intensity received, which can inhibit leaf growth and lead to decreased biomass accumulation. Yudina et al. (2023) found that increasing the duration of lighting can stimulate dry weight accumulation. In this study, a closer distance of 35 cm for the LED light source (SL-35) stimulated more significant leaf growth. Consequently, lettuce plants with a high leaf density and thickness tend to exhibit higher biomass accumulation.

In addition to light, the nutrient content is also essential for biomass accumulation. Plants require a sufficient supply of nutrients to grow and develop optimally. Fresh shoot weight reflects accumulated photosynthetic products and the water content of the plant. An adequate supply of nutrients stimulates photosynthesis, thereby supporting plant growth and biomass accumulation. Plants with an adequate nutrient supply stimulate photosynthesis. Meng et al. (2021) reported that deficiencies in nutrients, including phosphorus, negatively affect photosynthetic performance by reducing chlorophyll content. However, nitrogen is a nutrient that significantly affects photosynthetic performance. Mu et al. (2021) reported that the function of several components involved in photosynthesis declines due to nitrogen deficiency. The impact of nitrogen deficiency on photosynthesis has been demonstrated in leafy vegetables such as cabbage and lettuce

(Song et al., 2021). Furthermore, nutrient availability is essential for root growth in plants. Roots absorb nutrients from the growing medium. Ma et al. (2020) reported that nutrient deficiency can disrupt root development in the early stages of plant growth. Nutrient deficiency has been shown to cause inhibited root growth in several plants, including spinach (Ma et al., 2022). Furthermore, the amount and frequency with which nutrients are applied significantly affect the growth of red lettuce.

This study showed that 3rd split fertilization treatment led to higher fresh and dry weights. This suggests that the gradual application of fertilizer has a positive effect, as it provides plants with a continuous supply of nutrients according to physiological need. However, applying fertilizer that does not support the specific needs and growth stages of the plant can lead to nutrient deficiencies in red lettuce, which directly affects biomass accumulation. The SL-35:3rd treatment combination had a positive effect on the fresh and dry weights of all plant components, enhancing biomass accumulation. This indicates that in indoor cultivation systems using LED lighting, it is essential to maintain an optimal light distance and ensure proper nutrient management by fertilising at frequencies aligned with the physiological needs of the plant. In indoor red lettuce cultivation, optimising productivity in urban areas requires effective integration of light intensity and nutrient availability. According to Liang et al. (2022) the synchronized interaction between sufficient light intensity and nutrient availability promotes efficient assimilate distribution between shoots and roots, resulting in balanced vegetative growth. Therefore, in indoor red lettuce cultivation, optimizing productivity in urban areas requires an effective integration of light intensity and nutrient availability to promote plant growth and development.

Microclimate effects on red lettuce

Indoor farming conditions cause different microclimates. Fluctuations between air temperature and humidity in the morning, midday, and evening are shown in (Fig. 8).

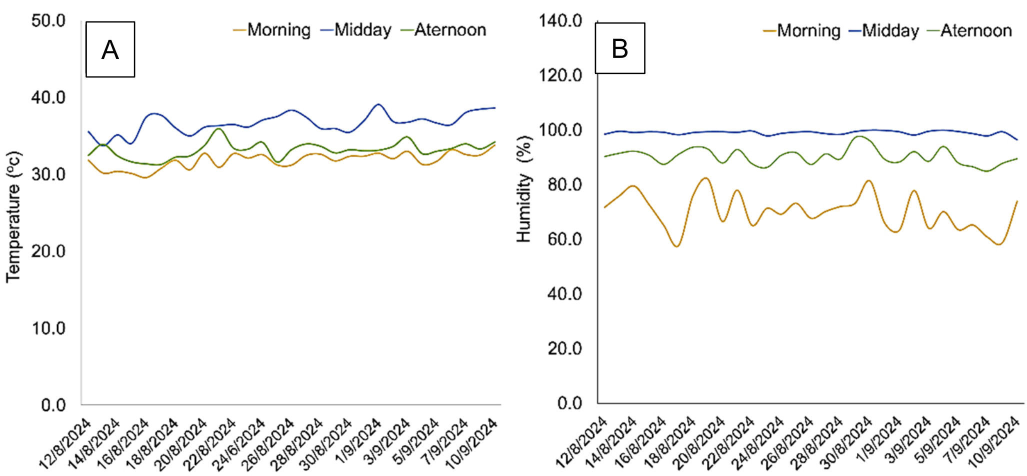

Figure 8. Temperature (A) and humidity (B) as affected by source light distance in indoor cultivation system.

Furthermore, the microclimate conditions inside the research room demonstrated consistent daily climate trends during the observation period. The air temperature ranged from 29 °C in the morning to 40 °C at midday with an average daytime temperature of 36 °C and afternoon temperature of 29 °C (Fig. 8, A) tends to be higher during the day. These trends are typical of temperature fluctuations in the tropics, with maximum temperatures occurring during the day. While relative humidity (Fig. 8, B) varied between 70% and 100% with average midday and humidity values of approximately 85–95% and 75–80%, the highest air humidity values are recorded at midday (Fig. 8, B).

Microclimate conditions are one of the factors that influence indoor cultivation systems. The results of this study indicated that the air temperature and relative humidity in the growth chamber were within the optimal range for supporting lettuce development. The visual appearance of red lettuce leaves is also influenced by environmental factors, including temperature, humidity, and nutrient availability (Lee et al., 2022). According to Iqbal et al. (2022), stable temperature and humidity support photosynthesis efficiency and reduce physiological stress in plants. Excessive water loss through transpiration can be caused by too low humidity, while growth can be slowed by air temperatures that are too high (> 30°C). Thus, consistent temperature and humidity control support optimal morphological growth of lettuce under LED lighting. The microclimate conditions inside the research room demonstrated consistent daily climate trends during the observation period. Climatic conditions in the research room are necessary to support plant growth. A stable microclimate is essential in indoor cultivation systems, as enclosed environments are more sensitive to fluctuations in temperature and humidity. Optimal temperatures support enzymatic activity, while adequate humidity maintains cell turgor and transpiration efficiency (Moore et al., 2021).

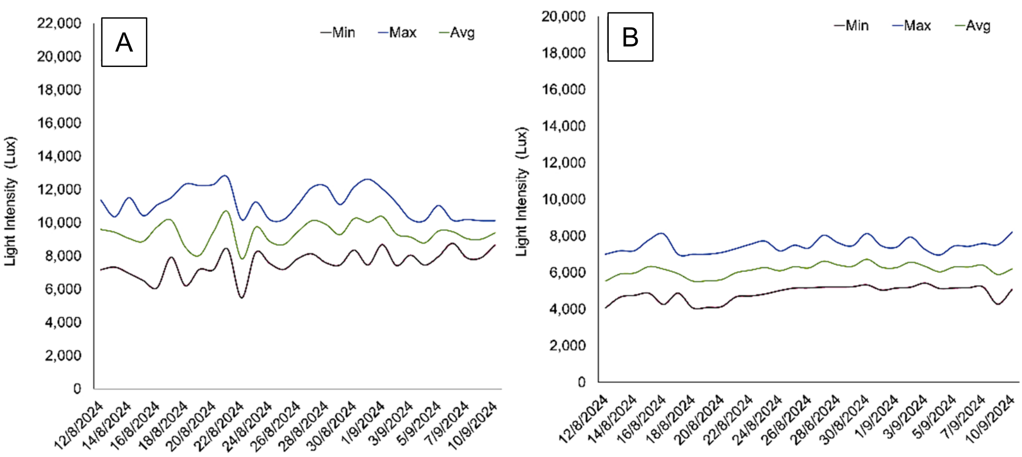

Figure 9. Light itensity as affected by source light distance 35 cm (A) and 50 cm (B) in indoor cultivation system

The closer the distance between the light source and the plant canopy, the higher the light intensity received (Fig. 9), which has the potential to enhance photosynthetic efficiency in indoor cultivation systems. In addition, light intensity is crucial for supporting photosynthesis, especially in crops like red lettuce, which rely on optimal lighting for the formation of pigments such as chlorophyll and anthocyanins (Sutuliene et al., 2022). Higher light intensity can stimulate chlorophyll production, thereby improving carbon assimilation, and promoting anthocyanin accumulation, which contributes to the red pigmentation associated with stress adaptation.

However, if increased light intensity is not accompanied by proper control of temperature and humidity, plants may experience heat stress or reduced transpiration efficiency, ultimately lowering the rate of photosynthesis (Paradiso & Proiette, 2022) Therefore, optimizing light intensity by adjusting the distance of the lamps is essential for supporting the physiological and morphological development of red lettuce under indoor cultivation. Therefore, optimizing light intensity by adjusting the distance of the lamps is essential for supporting the physiological and morphological development of red lettuce under indoor cultivation.

Leaf area estimation model

Leaf area estimation is used to estimate leaf area, which requires periodic non-destructive measurements. Morphological traits, which include leaf length, width, and thickness, are measured to create the predictors. The morphological characteristics used in this study were leaf length (P), leaf width (L), and leaf width multiplied by leaf length (LW). Leaf width is measured based on the largest width of the leaf, vertical to the selected leaf stalk. The destructive leaf area estimation method is carried out by exploring several regression models. The regression model is based on a comparison of the coefficients of determination (R^2) of various regression models. This study demonstrates that the zero-intercept quadratic linear model is the most accurate, as indicated by the R^2 value, for both single predictors (P or L) and multiple predictors (P×L). Therefore, the more precise model for estimating the leaf area of red lettuce is a zero-intercept quadratic linear regression with numerous predictors (P×L) (Fig. 10). The leaves are one of the most valuable parts of the red lettuce plant, as they are edible and can be used in various culinary applications. Moreover, leaves are also organs that have many functions in the growth process. Leaves are organs that function to absorb light energy and convert it into chemical energy for the photosynthesis process (Zhang et al., 2022b). This critical function of leaves is fundamental to understanding the rate at which leaf blades expand. This rate can only be accurately determined by continuously measuring the same leaves while the leaves are still alive.

Daily leaf area data collection on individual leaves cannot be done using a standard leaf area meter, but leaf blade length (P) and width (L) can be measured daily on the same leaf without having to cut the leaf stalk. The length and width of red lettuce leaves are essential factors in determining leaf area predictors, particularly for plants with pinnate leaf morphology. These predictive factors have also been successfully applied to other types of plants with comparable leaf shapes, such as butterhead lettuce (Muda et al., 2024) and Swiss chard (Ria et al., 2023). This study consistently demonstrated that zero-intercept quadratic linear regression with multiple predictors (P×L) is an accurate predictor of red lettuce leaf blade area. This model is highly relevant for use in studies of plant growth, physiological analyses, and monitoring of plant responses to various environmental and agronomic treatments. These findings support the use of an efficient and practical method for measuring leaf area indirectly in field applications, particularly in large-scale settings or plant growth research. This mathematical model greatly facilitated the rapid collection of plant morphological data, without the need for an expensive digital leaf area meter.

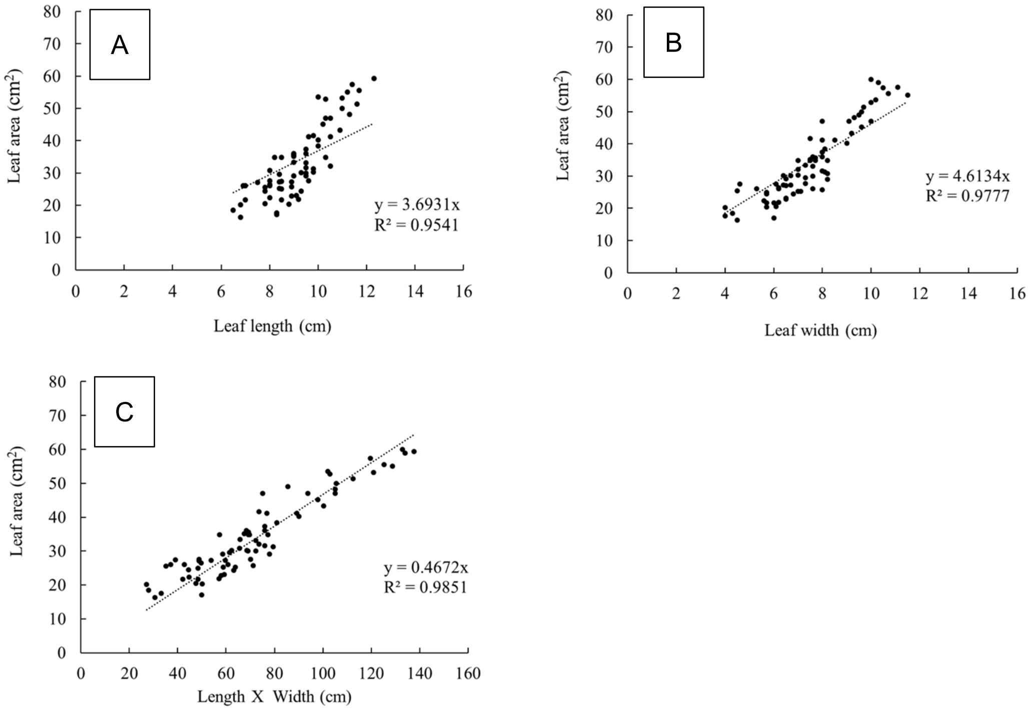

Figure 10. Estimation of leaf area using a single trait with regression linier zero intercept quadratic model.

CONCLUSIONS

Indoor cultivation of red lettuce using LED lighting at a 35 cm distance (SL-35) effectively promotes plant growth and improves key morphological traits. The third split fertilization was identified as optimal for enhancing leaf, stem, and root development. Additionally, a quadratic linear regression model without an intercept, based on leaf length and width (P × W) provided the most accurate leaf area estimation. These findings provide practical implications for urban farming, especially indoor systems using purple LED lighting by optimizing light distance and fertilization strategy to enhance growth efficiency and morphology traits.

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