



Morpho-physiological and yield responses of green lettuce to growing media volumes in urban cultivation

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Abstract: Urban agriculture requires efficient production in restricted spaces, making growing medium volume a critical determinant of lettuce performance. This study evaluated the effects of three medium volumes on the morpho-physiological responses and yield of green lettuce (*Lactuca sativa* L.). The experiment was arranged in a randomized complete block design with three polybag sizes, i.e. 15 × 20 cm (V1), 20 × 25 cm (V2), and 25 × 30 cm (V3), each treatment replicated four times, with six plants per replication. The smallest medium volume (V1) increased midday soil temperature by 34°C and leaf temperature by 33°C, while reducing soil moisture by 20% relative to V3, indicating greater heat and water stress. These conditions reduced canopy development, resulting in 61.6% fewer leaves, smaller leaf dimensions (leaf area decreased by 74.8%), and lower yield (-76.7%). In contrast, the largest medium volume (V3) improved the root zone microclimate and plant water status, producing the highest vegetative growth and yield. Leaf area was reliably predicted non-destructively using the linear model ($Y = 0.8019x$) (where (Y) is leaf area and (x) is leaf length × width), with a coefficient of determination ($R^2 = 0.9882$). Overall, increasing growing medium volume enhanced root zone conditions, leaf area development and final yield. Small volumes remain usable in urban systems if supported by adequate irrigation to maintain soil moisture.

1. Introduction

Urban agriculture increasingly relies on container-based cultivation (pots, polybags, or modular units), where the binding constraint is often finite root-zone or growing medium volume rather than land area; therefore, performance must be optimized not only per unit area but also per unit cultivation volume (e.g., yield per growing media volume or per unit substrate volume). Land scarcity requires food production systems to adapt to constrained environments such as rooftops, balconies, and vertical structures (Harada and Whitlow, 2020). In a 20 ft shipping container vertical farm (12 m² total footprint; 7 m² growing area), reported harvests reached 55 lettuce plants per week with a mean fresh

mass of 0.118 kg plant⁻¹ (± 1.85 kg m⁻² week⁻¹), illustrating how high output depends on the ability of a small, fixed cultivation volume to sustain a stable and productive root zone environment (Carrasco *et al.*, 2024). Root zone conditions are highly consequential for lettuce; for example, a root-zone temperature of 35°C (vs 25°C) was reported to suppress growth, while even relatively small shifts ($\pm 3^\circ\text{C}$) can measurably alter productivity, underscoring why container/root-zone limitation is a central design problem in urban systems (Levine *et al.*, 2023). The arrangement of pot size or growing medium volume is a crucial factor because it strongly influences the number of plants that can be cultivated in a limited area. Suboptimal growing medium volume directly affects root development, water retention, nutrient availability, and the overall physiological performance of the plants.

Green lettuce (*Lactuca sativa* L.) is one of the most widely produced leafy vegetables in urban agriculture due to its short cropping cycle, high market demand, and adaptability to container-based production systems (Ria *et al.*, 2025 a). Green lettuce is commonly grown in small media (e.g., net pots) in hydroponic systems (Ezziddine and Liltved, 2021). However, hydroponic systems can entail higher installation and maintenance costs, making conventional substrates in containers an accessible alternative for household and community growers (Nitu *et al.*, 2024). Nevertheless, lettuce growth is highly sensitive to root-zone restriction because its shallow, relatively fine root system depends on an adequately sized substrate volume to buffer water and nutrient supply; when the rooting volume is limited, root exploration and water uptake decline, the root zone dries and warms more rapidly, stomatal conductance and leaf expansion are reduced, and fresh leaf yield decreases (Levine *et al.*, 2023). Reducing growing medium volume can restrict root expansion, reduce water holding capacity, alter water use dynamics, and shift biomass allocation between shoot and root compartments (Holz *et al.*, 2024; Gustiar *et al.*, 2025). Under typical urban practice where growing media volume downsizing is used to increase planting density these constraints can translate into measurable yield penalties and reduced resource use efficiency unless the minimum viable volume and its management requirements are known.

Previous studies have shown that shoot morphology, leaf expansion, root architecture, and

physiological traits such as water-use efficiency and photosynthetic capacity are strongly influenced by root-zone restriction (Poorter *et al.*, 2012). However, previous studies have discussed container/root zone restriction in general, but few have quantitatively integrated soil temperature (root-zone microclimate), leaf area development, and fresh leaf yield to explain how limited growing medium volume constrains lettuce performance in growing media volume urban cultivation. Such research is essential to determine the most appropriate growing media volume, improve resource-use efficiency, and ensure that plant performance remains optimal in small-scale or modular production systems. Therefore, this study aims to evaluate the morpho-physiological responses of green lettuce grown under different growing medium volumes in order to identify the minimum volume that can still maintain optimal growth and yield.

2. Materials and Methods

The experiment was conducted in an open field located in Jakabaring, Palembang, South Sumatra, Indonesia (104°46'44" E; 3°01'35" S) from September-November 2025. The site is situated in a lowland tropical climate region. The mean environmental conditions, including temperature, rainfall, and relative humidity during the study period, are presented in figure 1. The plant material used was green lettuce (*Lactuca sativa* L.) cv. Grand Rapids. Prior to sowing, the seeds were soaked in warm water at 60°C, following a previous study, to accelerate germination (Ria *et al.*, 2025 b). After soaking, the seeds were directly sown into seedling trays filled with a mixture of ultisol (loam textured soil) and chicken manure at a 2:1 (v/v) ratio.

At 14 days after planting (DAP), the seedlings were transplanted into polybags of different sizes: 15 × 20 cm (V1≈1.43 L), 20 × 25 cm (V2≈3.18 L), and 25 × 30 cm (V3≈5.97 L). The experiment was arranged in a randomized complete block design (RCBD), in which each treatment was replicated four times, and each replication consisted of six plant samples, resulting in a total of 72 experimental plants. One seedling was planted per polybag. At 7 days after transplanting (DAT), each plant was supplied with a liquid organic fertilizer containing 10.04% organic C, 2.05% (N + P₂O₅ + K₂O), 0.54% organic N, pH 5.44, total Fe 332.21 ppm, Mn 38.8 ppm, B 74.20 ppm, and Mo 2.39 ppm.

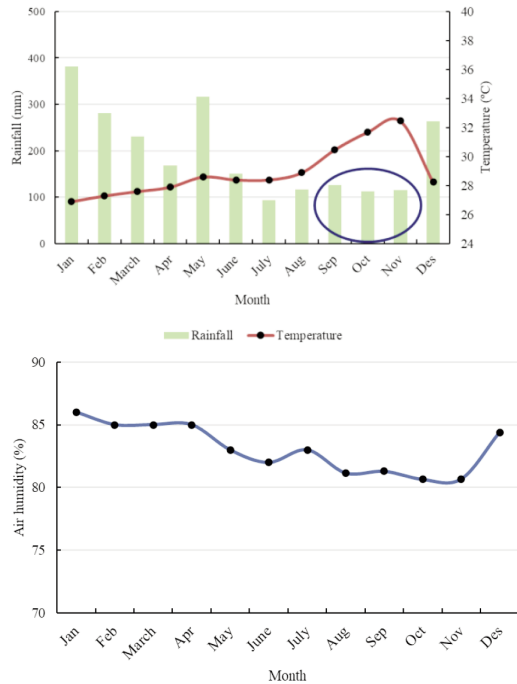


Fig. 1 - Dynamics of temperature, rain fall, and air humidity during the observation period.

Fertilization was conducted once a week in the late afternoon. Each plant received 100 mL of a diluted liquid organic fertilizer solution at a concentration of 2 mL L⁻¹. Plant watering was carried out once daily in the late afternoon.

Data collection

Data collection was carried out from 10 DAT to 35 DAT, and harvesting was conducted at 42 DAT. Air temperature was measured using a digital thermometer. Leaf temperature was measured using a FLIR C3-X thermal camera, with thermal images captured from above the leaf canopy at a distance of ± 50 cm from the top of the plants. Soil temperature and soil moisture were measured simultaneously using a digital environmental thermometer and a Lutron PMS-714 soil moisture meter, respectively, at a depth of ± 5 cm and a distance of ± 7 cm from the plant stem. Measurements were taken on a single day at 2-hour intervals from 08:00 to 16:00.

Growth- and yield-related parameters were also recorded, including canopy area, leaf area, and estimated leaf area. Canopy area was quantified using the Easy Leaf Area application (Easlon and Bloom, 2014), while actual leaf area was measured using LIA32 software from Nagoya University. Leaf area estimation was performed using an exponential regression model based on leaf length and width,

and a zero-intercept linear regression model using leaf length $\times$ width (cm²). Plant dry weight was determined after drying plant samples in a forced-air oven at 105°C for 24 hours.

Data analysis

All collected data were analyzed using analysis of variance (ANOVA). For variables showing significant treatment effects, mean separation was performed using the Least Significant Difference (LSD) test at a significance level of $P < 0.05$. All statistical analyses were carried out using RStudio software.

3. Results

Root-zone microclimate responses

Measurements of soil temperature and soil moisture indicated that growing medium volume altered the root-zone microclimate (Fig. 2). Across

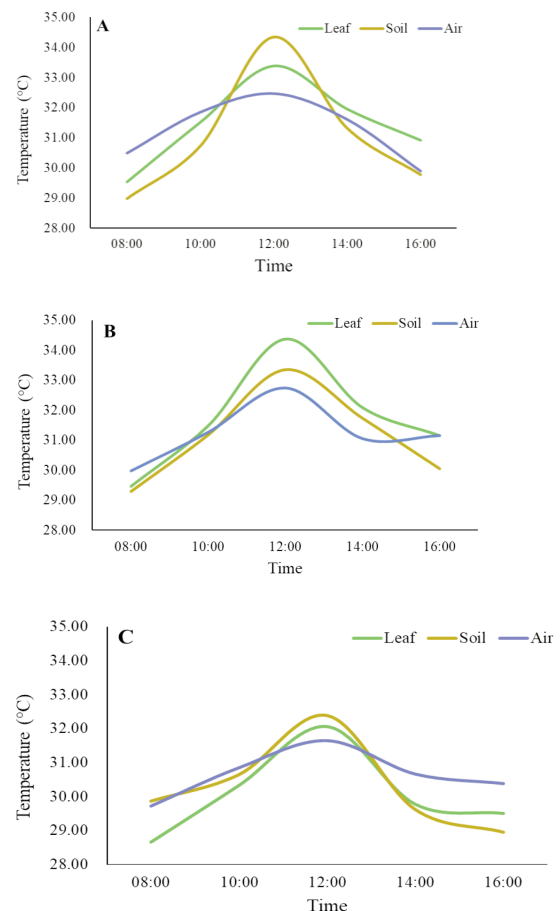


Fig. 2 - Fluctuations in leaf temperature, soil temperature, and air temperature at different volume levels. Small (A), medium (B), and big (C), in green lettuce plants.

treatments, soil temperature showed a clear inverse association with soil moisture: when soil temperature increased from approximately 27°C to 32°C, soil moisture declined (Fig. 3). This pattern suggests that smaller media volumes provide limited buffering capacity against heat gain and water loss, resulting in faster drying of the root zone. In contrast, larger media volumes maintained more stable soil conditions, which likely improved the continuity of water availability for root uptake.

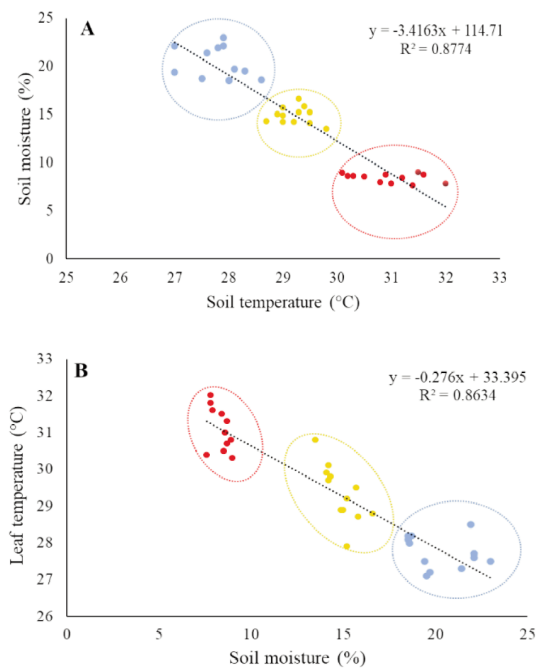


Fig. 3 - Relationship between soil temperature and soil moisture (A); and between soil moisture and leaf temperature (B) in green lettuce plants at different growing media volumes.

Leaf temperature and water relations

Leaf temperature dynamics further reflected differences in root-zone buffering among media volumes. In the morning, air temperature was higher than both leaf and soil temperatures; however, around midday (12:00), leaf and soil temperatures increased more sharply than air temperature, particularly in smaller polybags. This coincided with lower soil moisture and was accompanied by higher leaf temperatures (Fig. 2), indicating tighter coupling between root-zone water status and canopy thermal responses. Elevated leaf temperature can accelerate transpiration and increase the risk of transient tissue dehydration when root water supply is constrained by limited media volume. Consequently, plants

grown in smaller polybags are more prone to rapid turgor loss and midday wilting. Conversely, plants grown in larger polybags maintained lower and more stable leaf temperatures, consistent with better root-zone moisture retention and improved capacity to sustain plant water status during peak thermal load.

Vegetative growth and canopy development

Growing medium volume strongly influenced vegetative development. Plants cultivated in the largest media volume (V3) consistently produced more leaves than those in V1 and V2, and the differences became increasingly pronounced with plant age (Fig. 4). Canopy development showed a similar response: canopy area increased over time in all treatments, but the rate of canopy expansion was substantially higher in the larger media volumes. The greater canopy area in V3 reflects both higher leaf number and larger leaf size, which likely enhanced light interception and, consequently, photosynthetic potential during vegetative growth (Fig. 5).

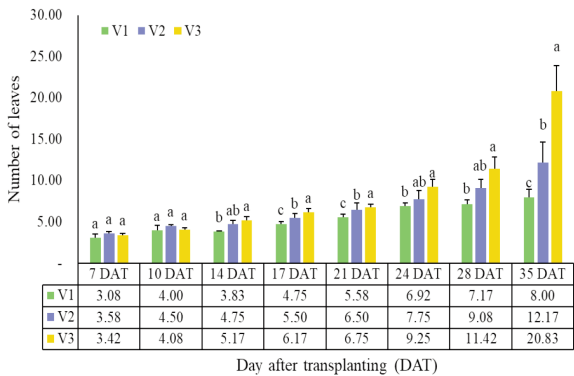


Fig. 4 - Number of leaves of green lettuce plants at different growing media volumes.

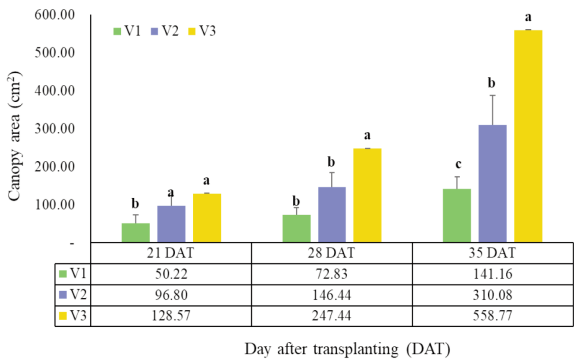


Fig. 5 - Canopy area of green lettuce at various growing media volumes.

Leaf morphology and area relationships

Leaf length and leaf width increased with days after transplanting (DAT) across all treatments (Fig. 6). Nevertheless, plants grown in V3 exhibited the greatest increase in leaf size throughout the observation period, whereas V1 produced the smallest leaves, consistent with limitations in root space and reduced water retention capacity of the smaller media volume (Fig. 7). The relationships among leaf length, leaf width, and leaf area showed varying R^2 values (Fig. 8). The highest $R^2 = 0.9882$ value was found in the relationship between leaf length $\times$ leaf width and leaf area. The very tight clustering of data points around the regression line

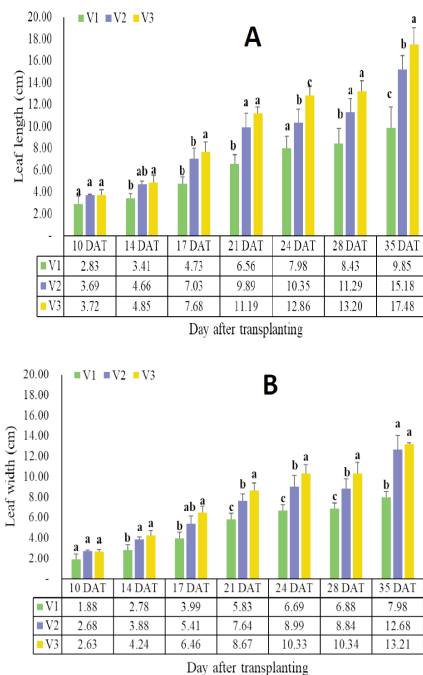


Fig. 6 - Growth of leaf length (A) and leaf width (B) of green lettuce at various growing media volumes.



Fig. 7 - Comparison of shoots and roots of green lettuce at different volumes.

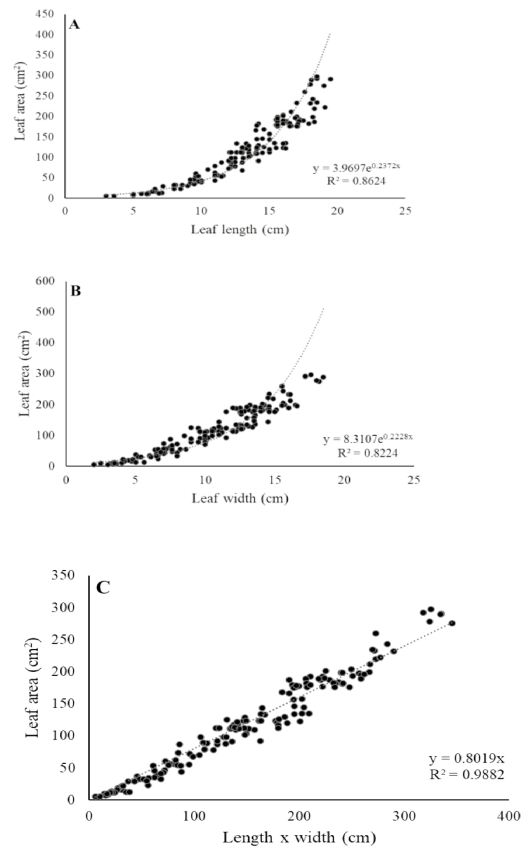


Fig. 8 - Estimation of green lettuce leaf area by relating leaf length and leaf area (A); leaf width and leaf area (B); and leaf length $\times$ leaf width and leaf area (C).

confirms that this linear model has very high accuracy across the full range of observed leaf sizes. These results indicate that although leaf length and leaf width individually are already sufficient to estimate leaf area, their combination in the form of length $\times$ width produces an estimator that is both more precise and simpler (requiring only a single multiplication constant). Thus, the linear equation based on length $\times$ width is highly promising as a



practical and non-destructive method.

Yield components and biomass allocation

Increasing growing medium volume significantly improved yield components and leaf morphophysiological traits (Table 1). The largest media volume produced the highest shoot and leaf fresh weight, leaf dry weight, leaf number, and total leaf area, and these values were consistently higher than those obtained in medium and small media volumes. In contrast, leaf water content did not differ significantly among treatments, suggesting that it was relatively insensitive to changes in media volume under the conditions of this study. Overall, the results indicate that enlarging the root zone primarily promoted biomass accumulation and leaf area development rather than altering tissue water

content.

Medium volume also affected biomass allocation to stems (Table 2). Larger media volumes were associated with higher stem fresh weight, stem dry weight, and stem length, which are consistent with improved water and nutrient supply supporting photosynthetic production and subsequent allocation of assimilates to structural tissues. The absence of significant differences in stem water content among treatments further indicates that increases in stem mass were driven mainly by dry matter accumulation rather than by greater tissue hydration. Consistent with these patterns, larger media volumes enhanced both the root and shoot growth, producing more balanced and vigorous plant development, whereas limited media volume restricted the expansion of both root system and shoot biomass (Table 3).

Table 1 - Fresh weight, dry weight, and leaf morphophysiological components of green lettuce at various growing media volumes

Treatment	Shoot fresh weight (g)	Number of leaves	Leaf fresh weight (g)	Leaf dry weight (g)	Leaf water content (%)	Total leaf area (cm ²)
V1	36.69 c	12.50 c	30.76 c	2.82 b	90.61 a	1023.12 c
V2	93.12 b	19.11 b	72.43 b	5.42 ab	92.67 a	2558.94 b
V3	170.41 a	28.44 a	131.77 a	9.89 a	92.40 a	3868.95 a
LSD 0.05	42.05	5.78	30.49	7.63	5.48	761.81

Means followed by different letters are significantly different according to the LSD test at the 0.05 significance level.

Table 2 - Fresh weight, dry weight, and stem morphophysiological components of green lettuce at various growing media volumes

Treatment	Stem fresh weight (g)	Stem dry weight (g)	Stem water content (%)	Stem length (cm)
V1	5.93 c	0.56 b	88.48 a	8.69 c
V2	20.69 b	1.13 ab	94.63 a	17.71 b
V3	38.08 a	1.85 a	95.23 a	26.04 a
LSD 0.05	11.47	0.82	11.94	5.63

Means with different letters within the columns are statistically different according to the LSD test ($p \leq 0.05$). ns, non-significant, * significant difference at $P \leq 5\%$, and ** significant difference at $P \leq 1\%$. CV= Coefficient of variation.

Table 3 - Fresh weight, dry weight, and root morphophysiological components of green lettuce at various growing media volumes

Treatments	Root length (cm)	Shoot root ratio	Root fresh weight (g)	Root dry weight (g)
V1	7.76 c	6.06 b	7.06 c	0.38 c
V2	18.30 b	8.12 ab	11.49 b	0.84 b
V3	25.00 a	10.65 a	16.99 a	1.67 a
LSD 0.05	6.45	4.52	3.7	0.21

Means with different letters within the columns are statistically different according to the LSD test ($p \leq 0.05$).

4. Discussion and Conclusions

Effect of growing media volume on soil microclimate

An increase in soil temperature in small polybags was accompanied by a decrease in soil moisture, as reflected by the negative linear relationship between these two variables. This consistent pattern indicates that restricted media volume accelerates water loss through evaporation and exacerbates fluctuations in substrate moisture in container-grown plants (Witcher *et al.*, 2020). As soil moisture declines, the flow of water from roots to leaves is reduced, while increasing radiation and air temperature continue to drive a steeper water potential gradient between the leaves and the atmosphere. Consequently, the transpiration rate and leaf temperature increase. In small media volumes, the root zone tends to exhibit leaf and soil temperatures above air temperature because of the low heat capacity and low water content, making it prone to overheating (supra-optimal root-zone temperatures) (González-García *et al.*, 2023).

Physiologically, the combination of high leaf temperature and declining soil water availability causes plants to experience simultaneous heat and water stress. Previous studies have shown that the interaction between restricted root volume due to limited media and water stress can reduce stomatal conductance, decrease the photosynthetic rate, and accelerate the decline in turgor pressure, making plants more prone to wilting during the day (Wu *et al.*, 2022; Asargew *et al.*, 2023). This was clearly evident in the smallest media volume treatment (V1), where the high leaf temperature was not compensated by water supply from the roots because of the narrow root zone and limited water content.

In contrast, in large polybags, soil and leaf temperatures tended to be lower and showed smaller fluctuations. Larger media volumes have higher water storage capacity and heat capacity, enabling them to buffer daily temperature changes and maintain soil moisture for longer. Growing media with larger volume or substrates with good water retention capacity can stabilize root-zone temperature, reduce thermal stress, and help maintain leaf turgor in horticultural crops (Irshad *et al.*, 2024). In this study, differences in growing media volume primarily acted as a “microclimate buffer” around the roots and canopy, which in turn

determined plant water status, stomatal activity, and the physiological performance of green lettuce.

Effect of growing media volume on lettuce morphology

Significant differences in leaf number and canopy area of lettuce indicate that more favorable microclimatic conditions and resource availability occurred in the largest media volume (V3). This promoted leaf meristem activity and prolonged the vegetative growth phase. Root volume restriction reduces the rate of new leaf formation and decreases leaf size as a result of reduced photosynthetic rate and increased growth-inhibiting hormonal signals from stressed roots (Triyono *et al.*, 2020; Nasih *et al.*, 2025). The results of this study are consistent with those findings, as the V1 treatment (smallest volume) exhibited inhibited shoot growth.

Lettuce leaf length and width increased consistently with days after transplanting; however, the rate of increase was strongly influenced by media volume. Leaf elongation and expansion are highly sensitive to root-zone space and the availability of water and nutrients (Omar *et al.*, 2023). The narrow and easily dried root-zone environment in V1 triggered an adaptive response in the form of reduced leaf surface area to minimize water loss through transpiration. In contrast, the wider and moister root zone in V3 enabled leaf cells to maintain turgor, allowing optimal cell expansion and leaf elongation (Li *et al.*, 2022).

Effect of growing media volume on yield and biomass of lettuce

Increasing the volume of the growing media had a significant effect on yield components and biomass of lettuce. The V3 treatment consistently produced the highest shoot and leaf fresh weight, leaf dry weight, number of leaves, and total leaf area, whereas V1 gave the lowest values for almost all parameters. Interestingly, leaf water content did not differ markedly among treatments, indicating that differences in fresh weight were primarily determined by increased dry matter accumulation rather than changes in tissue water status. Lettuce and other leafy vegetables tend to increase shoot dry biomass without drastically altering leaf water content (Marutani *et al.*, 2021).

The stem functions as an important “sink” for additional photosynthates produced under larger

media volumes. The improved water and nutrient availability in V3 support a higher photosynthetic rate, thereby increasing carbohydrate supply to storage organs such as the stem (Sarkar *et al.*, 2021). The strengthening of stem and shoot sinks under adequate root volume conditions is consistent with the concept that root volume restriction tends to reduce shoot growth and alter root-shoot biomass partitioning in various plant species (Antunes *et al.*, 2024).

On the other hand, root system data show that increased media volume not only improved shoot growth but also enhanced root development (Table 3). The V3 treatment produced the greatest root length and root biomass, along with the largest shoot biomass. In contrast, V1 simultaneously restricted both components. The size of the growing media determines the volume of root exploration and, through this, influences the plant's capacity to absorb water and nutrients, which ultimately determines shoot growth and yield (Gallegos *et al.*, 2020). In this study, increasing media volume acted to enhance both source capacity (roots as water and nutrient absorbers) and sink capacity (shoots and stems as photosynthate storage organs) (Table 3).

Leaf area estimation

In this study, the relationships between leaf length, leaf width, and leaf area were measured to estimate leaf area using a non-destructive, coefficient-based approach. Similar studies have been conducted on various leafy vegetables, including Swiss chard (Ria *et al.*, 2023), tatsoi (Kartika *et al.*, 2021), and chaya (Gustiar *et al.*, 2024). The present results show that the combination of length $\times$ width produced the highest coefficient of determination (R^2) with leaf area. The tight clustering of data points around the regression line confirms that this simple linear model based on length $\times$ width is a highly precise estimator of leaf area across the full range of leaf sizes observed. These findings are consistent with previous reports showing that the leaf area of lettuce and other leafy vegetables can be accurately predicted using linear regression models based on leaf length and width, with R^2 values above 0.95 (Rahimikhoob *et al.*, 2023). Practically, such a model offers advantages because it is non-destructive, easy to apply in the field or greenhouse, and highly useful for monitoring leaf morphological responses to various treatments, including different

growing media volumes.

This study shows that increasing growing media volume improves the root zone microclimate (lower soil and leaf temperatures and higher moisture retention), thereby supporting vegetative growth and enhancing yield components. However, the smallest media volume (V1) can still be recommended for urban farming because it is more space-efficient and allows higher planting density per unit area. The use of a minimum media volume is feasible when accompanied by adequate irrigation management particularly more frequent and well-timed watering to maintain substrate moisture and reduce the risk of midday wilting. Since this study was performed under a single environmental condition and involved only one lettuce cultivar, the generalizability of the findings may be limited. Further research under diverse climatic conditions, using different cultivars and irrigation regimes, is therefore necessary to verify the robustness and broader applicability of the results.

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