

Optimizing Seedling Density per Planting Hole of Lettuce (*Lactuca sativa* L. var. *longifolia*) in a Deep Flow Technique Hydroponic System

I Gede Febriana, Gede Wijana[✉], I Made Sukewijaya, Ida Ayu Putri Darmawati, and
Ni Luh Made Pradnyawathi

Agroecotechnology Study Program, Faculty of Agriculture, Udayana University, Denpasar, Indonesia

[✉]Corresponding author email: wijana07@yahoo.com

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Abstract. Market demand for baby romaine lettuce (*Lactuca sativa* L. var. *longifolia*) continues to rise alongside the public's growing preference for healthy lifestyles, yet its productivity still needs improvement. This study aims to evaluate the effect of seedling density per planting hole on the growth and yield of baby romaine lettuce using the Deep Flow Technique (DFT) hydroponic system and to determine the optimal number of seedlings. The experiment was conducted in a greenhouse in Pelaga Village, Bali, using a completely randomized design (CRD) with a single factor comprising five seedling densities (1, 2, 3, 4, and 5 seedlings per hole) and six replications, totaling 120 plants. Growth and yield parameters were analyzed using ANOVA and LSD at a 5% significance level, with regression applied to fresh shoot and crop weight. Results showed that increasing the number of seedlings significantly affected plant height, stem diameter, chlorophyll content, fresh and dry shoot weight, and total fresh biomass. Shoot fresh weight increased linearly ($Y = 97.86 + 11.71X$; $R^2 = 0.975$), while crop fresh weight followed a quadratic trend ($Y = 70.64 + 8.10X - 1.00X^2$; $R^2 = 0.863$), with the optimal yield obtained at three seedlings per hole. It is concluded that planting three seedlings per hole offers the best outcome in terms of both quality and quantity in a DFT hydroponic system.

Keywords: baby romaine lettuce; DFT hydroponics; optimal crop weight; seedling density; shoot biomass

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a widely consumed seasonal horticultural vegetable in Indonesia, enjoyed fresh as salad or lalapan. Market demand has increased in line with healthier dietary trends. Baby Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) has gained popularity due to its crunchy texture, slightly sweet taste, and dense, oval heads. Despite growing consumer interest, domestic production often fails to meet this demand (Hafidz, 2023). Traditional cultivation during the rainy season faces high humidity, which promotes pest and disease incidence; farmers frequently resort to chemical pesticides (Sulistiyana, 2016), posing environmental and health concerns.

Hydroponic systems provide a sustainable alternative by eliminating soil use and controlling environmental variables. These systems reduce pesticide reliance and produce high-quality vegetables. Baby Romaine is particularly well-suited to hydroponic cultivation (Novitasari, 2020),

aligning with consumers' preference for fresh, healthy produce (Febrianti et al., 2019). Among hydroponic systems, the Deep Flow Technique (DFT) is promising—it maintains a constant ± 5 cm of nutrient solution in PVC channels, offering stable nutrition supply, energy efficiency, and resilience during power outages (Sugianto et al., 2024).

Nutrients are vital in hydroponics, providing all essential macronutrients and micronutrients in a soluble form (Purbajanti, 2017). However, optimal nutrient concentration varies among crops; for example Sipayung et al., (2025) found 1000 ppm optimal for pakcoy but not significantly different for green lettuce. Besides nutrient delivery, seedling density per planting hole is crucial. Higher density can boost total yield per area but may intensify plant competition (Jamaludin, 2018). Balancing density to enhance productivity without compromising quality is essential, especially for high-value crops like baby Romaine.



In addition, international studies on hydroponic plant density have consistently shown that density-driven changes in biomass allocation, light interception, and nutrient competition follow general resource-competition theory, with several meta-analyses reporting that increased density enhances total yield per unit area but reduces individual plant size due to intensified competition for light and root-zone resources. Synthesizing these findings underscores the theoretical basis for examining density effects in baby romaine lettuce and positions the present research within a broader global context of hydroponic optimization ([Anderson et al., 2017](#); [Bilotta et al., 2024](#); [Obia et al., 2018](#); [Poloviy et al., 2024](#); [Vega et al., 2022](#)).

Although numerous studies have examined plant density responses in hydroponic systems, most have focused on NFT or floating-raft systems and on species such as pakcoy, leafy lettuce, or other Brassicaceae crops, leaving a clear knowledge gap because no published research has evaluated the density–response of baby romaine lettuce specifically within a DFT system under high-elevation tropical greenhouse conditions. This study, therefore, contributes novel empirical evidence by quantifying how different seedling densities per planting hole influence growth and yield in a DFT system, providing information that is currently absent from the literature. This research aims to evaluate the impact of seedling density on the growth and yield of baby Romaine in a DFT hydroponic system, and to identify the optimal number of seedlings per planting hole.

METHODS

The study was conducted from November 2024 to January 2025 in a greenhouse at 950 m above sea level in Pelaga Village, Badung Regency, Bali, with an average temperature of 24 °C. The structure measured 9 × 6 m with lightweight steel framing and a UV-coated 200 µm plastic cover (UV14%).

A Deep Flow Technique hydroponic system comprising six 2.5-inch PVC pipes was used. Each pipe had planting holes spaced 20 cm apart, and a nutrient solution was circulated for 12 hours daily using a 3000 L/h capacity submersible pump (Armada 105). Rockwool-grown Rijk Zwaan baby Romaine seedlings (21 days old, 2–3 true leaves) were transplanted into net pots at densities of 1, 2, 3, 4, and 5 seedlings per hole. The experiment employed a one-factor completely randomized design (CRD) with six replications, each consisting of four plants, resulting in a total of 120 plants.

A complete AB Mix nutrient solution was applied at 1400 ppm with a pH range of 5.5–6.5. Nutrient concentration and pH were monitored regularly. Observations were made at 7, 14, 21, 28, and 35 days after transplanting (DAT). Growth variables included plant height, head circumference (crop), chlorophyll content (SPAD), stem diameter, root length, fresh shoot and root weight, total fresh weight, and dry weight after oven drying at 70–80°C for 12 hours.

In this study, the experimental unit for all analyses was the individual planting hole (net pot) containing the assigned number of seedlings. Treatments were randomly assigned to planting holes across all PVC pipes using simple randomization to ensure each hole had an equal probability of receiving any density level. The positions of treatments within each pipe were also randomized to minimize positional bias, and no blocking was required because the environmental conditions inside the greenhouse were homogeneous. However, two guard rows were established at both ends of each pipe to reduce potential edge effects.

All statistical analyses were performed using SPSS version 20. Prior to conducting ANOVA, data were evaluated for compliance with model assumptions, including normality of residuals using the Shapiro–Wilk test and homogeneity of variance using Levene’s test. When necessary, data were transformed to meet the assumptions of ANOVA, and the analysis reported exact p-values and degrees

of freedom for each tested parameter. Mean comparisons were conducted using the Least Significant Difference (LSD) test at $\alpha = 0.05$. For regression analyses of shoot fresh weight and crop fresh weight, model selection was based on biological relevance and goodness-of-fit criteria. Residual plots, normal Q-Q plots, and homoscedasticity checks were examined to verify regression assumptions, and 95% confidence intervals were calculated for all regression coefficients to ensure model reliability.

RESULTS AND DISCUSSION

Based on the analysis of variance, the treatment of seedling number per planting hole had varying effects—from non-significant to highly significant—on the growth and yield of baby Romaine lettuce in the Deep Flow Technique (DFT) hydroponic system. Parameters such as plant height, number of leaves, head circumference, stem diameter, shoot fresh weight, root fresh

weight, shoot dry weight, and total plant fresh weight showed highly significant differences. Meanwhile, chlorophyll content and crop fresh weight showed significant differences, and root length and root dry weight showed no significant difference.

As shown in [Table 1](#), the tallest plant height was observed in treatment J5 (5 seedlings), reaching 25.51 cm, significantly different from the other treatments. This indicates an adaptive response in plants to increased light competition resulting from higher population density. Conversely, J3 (3 seedlings) produced the shortest plants (15.38 cm). The highest number of leaves was recorded in J1 (1 seedling) with 6.21 leaves and decreased as the number of seedlings increased, suggesting that plants in denser populations allocate more energy to vertical growth rather than leaf formation ([Kurniawan, 2017](#)). The effect of seedling density on Baby Romaine Lettuce shoot fresh weight and crop fresh weight is shown in [Figure 1](#).

Table 1. Effect of Seedling Number per Planting Hole on Growth Parameters of Baby Romaine Lettuce at 35 Days After Transplanting (DAT)

Treatment	Plant Height (cm)	Number of Leaves (leaves)	Stem Diameter (mm)	Chlorophyll (SPAD)	Root Length (cm)
J1 = 1 plant	20.31 b	6.21 a	14.97 a	34.89 a	26.63 a
J2 = 2 plants	20.83 b	5.42 b	12.85 b	33.12 a	24.71 a
J3 = 3 plants	15.38 c	5.18 b	8.68 e	31.57 b	23.96 a
J4 = 4 plants	20.02 b	5.07 c	10.04 d	32.22 b	25.75 a
J5 = 5 plants	25.51 a	5.08 c	11.58 c	32.28 b	23.88 a
LSD 5%	0.91	0.34	0.65	2.01	2.62

Note: Different letters within the same column indicate significant differences at the 5% level based on LSD test.

The largest stem diameter was found in J1 (14.97 mm), while the smallest was in J3 (8.68 mm), indicating that lower density provides more space and nutrients, allowing the plant to develop stronger stems. The

highest chlorophyll content was also found in J1 (34.89 SPAD) and tended to decrease as plant density increased. This was likely due to competition for light, which reduced photosynthetic efficiency ([Coêlho et al.,](#)

2023; [Damayanti, 2017](#); [Elkins & van Iersel, 2020](#); [Nafiah et al., 2023a](#)). Although root length showed no significant difference, descriptively it declined from 26.63 cm in J1 to 23.88 cm in J5.

As shown in [Table 2](#), shoot fresh weight significantly increased in J5 (153.75 g) compared to J1 (99.38 g), indicating that total shoot biomass increased with a higher number of seedlings per planting hole. Root fresh weight and shoot dry weight also increased along with seedling number, while root dry weight showed no significant difference among treatments. The highest root dry weight was still observed in J5 (1.69

g), indicating a greater accumulation of total biomass in denser populations ([Rosyidah et al., 2024](#)).

Crop circumference showed a decreasing trend with increasing seedling number, from 22.79 cm in J1 to 9.66 cm in J5. The highest fresh crop weight, however, was observed in J3 (89.21 g). This indicates that although total yield per planting hole increased, the individual crop quality decreased due to more intense competition (Santoso, 2018). Total plant fresh weight was highest in J5 (187.17 g) and lowest in J1 (124.54 g), confirming that increased seedling number effectively boosts overall plant biomass.

Table 2. Effect of Seedling Number per Planting Hole on Fresh and Dry Weight of Baby Romaine Lettuce in DFT Hydroponic System

Treatment	Crop Fresh Weight (g)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Total Fresh Weight (g)	Shoot Oven-Dry Weight (g)	Root Oven-Dry Weight (g)
J1 = 1 plant	78.62 b	99.38 c	17.68 c	124.54 c	6.27 c	0.93 a
J2 = 2 plants	83.69 ab	125.51 bc	20.46 b	146.37 b	7.87 b	1.00 a
J3 = 3 plants	89.21 a	130.24 b	21.12 b	157.36 b	8.40 b	1.10 a
J4 = 4 plants	81.79 ab	148.16 ab	23.35 ab	179.55 ab	8.93 ab	1.37 a
J5 = 5 plants	73.73 b	153.75 a	25.42 a	187.17 a	9.06 a	1.69 a
LSD 5%	19,82	17,02	4,21	18,55	1,17	0,46

Note: Different letters within the same column indicate significant differences at the 5% level based on LSD test.

The observed reductions in chlorophyll content (SPAD values) and leaf number at higher seedling densities indicate that increased intra-canopy shading limited light availability for individual plants. Lower SPAD values in denser treatments likely reflect reduced photosynthetic capacity resulting from diminished light penetration, while the decline in leaf number suggests a strategic allocation shift toward height growth as plants compete vertically for light.

This is further supported by the smaller stem diameter in high-density treatments, which reflects elongation-driven competition rather than structural strengthening. Although direct measurements of canopy light interception or photosynthetic photon flux density (PPFD) were not taken in this study, the patterns observed strongly imply that light competition was a major driver of the biomass responses. Future studies should incorporate direct measurements of light

distribution within the canopy to quantify shading intensity and confirm its mechanistic role (Bae & Ko, 2025; Khan et al., 2019; Song et al., 2013; Vega et al., 2022; Zhen et al., 2019).

Regression analysis of fresh crop weight showed a quadratic pattern with the equation $Y = 70.64 + 8.10X - 1.00X^2$ ($R^2 = 0.863$), indicating that crop weight increased up to three seedlings per hole and then decreased due to competition for resources. The shoot fresh weight increased linearly with the equation $Y = 97.86 + 11.71X$ ($R^2 = 0.975$), indicating a significant total shoot biomass increase with additional seedlings (Damayanti, 2017).

Overall, increasing the number of seedlings per planting hole significantly increased total shoot biomass and total plant fresh weight, but reduced the individual crop quality. This is important for managing plant density in DFT hydroponic systems to maximize land and resource efficiency while balancing

quantity and quality of harvest (Jamaludin, 2018; Santoso, 2018).

This study has several limitations that should be acknowledged when interpreting its results. The experiment was conducted during a single growing season and used only one baby romaine cultivar, which may limit the generalizability of the findings across seasons or genotypes. The environmental conditions of the high-elevation greenhouse, including temperature stability and diffuse light quality, may differ from those in lowland or open-field hydroponic operations. Additionally, nutrient concentration was held constant at 1400 ppm throughout the experiment, preventing assessment of whether optimal density interacts with nutrient availability. These factors may constrain the external validity of the results, and caution should be exercised when extrapolating the recommended density to other climates, cultivars, or hydroponic management systems.

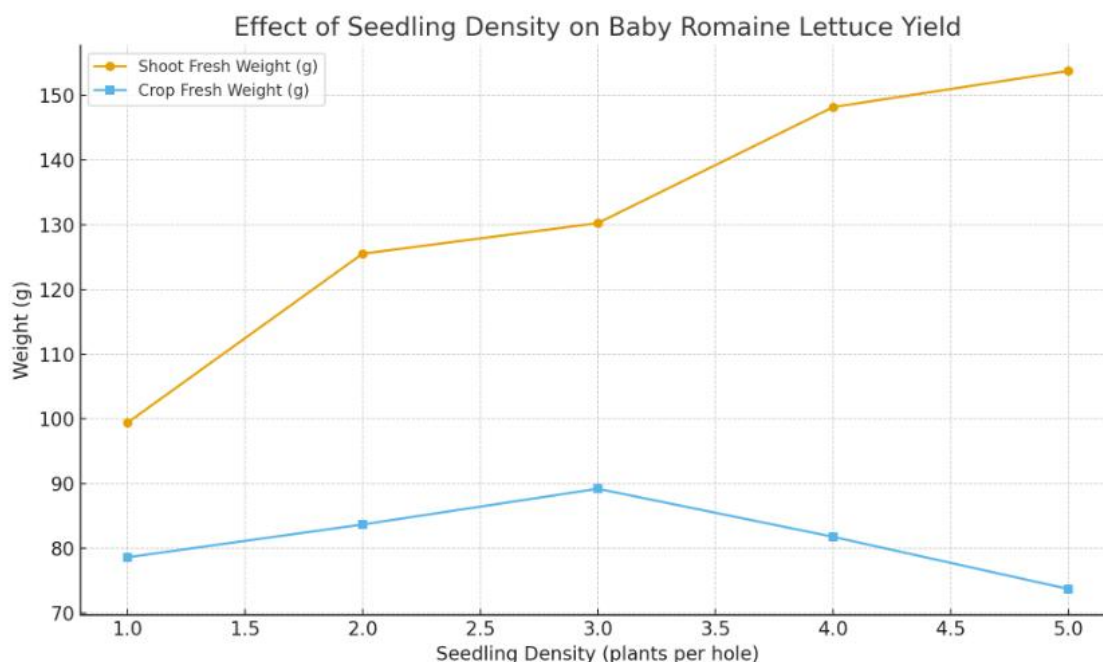


Figure 1. Effect of seedling density on Baby Romaine Lettuce yield

Future research should employ factorial experimental designs that simultaneously test the effects of seedling density, light intensity (PPFD), and AB Mix nutrient concentration to determine whether optimal plant density

shifts under different resource environments. Multisite and multi-season trials would also be valuable for validating the consistency of density responses under varying climatic conditions. Furthermore, integrating an

economic analysis that compares total yield per unit area with marketable head size would provide growers with practical decision-making tools. Studies incorporating canopy light mapping, competition indices, or photosynthetic efficiency measurements could also clarify the physiological mechanisms underlying density responses. These approaches will generate more robust, transferable recommendations for optimizing baby romaine production in DFT hydroponic systems ([Hartanti & Sulistyowati, 2022](#); [Nafiah et al., 2023b](#); [Suwitra et al., 2021](#)).

CONCLUSION

The number of seedlings per planting hole had a significant influence on the growth and yield of baby romaine lettuce cultivated in a Deep Flow Technique (DFT) hydroponic system. Increasing planting density from one to five seedlings per hole produced a linear increase in total shoot biomass, while crop fresh weight exhibited a quadratic response, with three seedlings per hole identified as the optimal density for balancing total yield and individual head quality. These findings demonstrate that strategic manipulation of plant density can enhance production efficiency in DFT systems, although the resulting trade-offs between biomass accumulation and head size must be carefully considered.

In practice, the adoption of the recommended density should be guided by market preferences for head circumference, post-harvest and processing requirements, and overall economic priorities, as growers may attach greater value to marketable head size than to biomass per planting hole. Therefore, the density of three seedlings per hole should be regarded as a technical benchmark rather than a universal commercial prescription. Before wider implementation, this recommendation should be validated across multiple seasons, locations, and greenhouse environments to ensure consistency under varying climatic and management conditions. Future assessments of marketability, harvest timing, and labor efficiency are also essential to

strengthen the practical relevance of this density strategy for commercial baby romaine production in DFT hydroponic systems.

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