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Sustainable hydroponics: Boosting red lettuce growth with photosynthetic bacteria and optimal transplanting

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ABSTRACT

Red lettuce growth under hydroponic conditions was significantly influenced by transplanting age, whereas the application of photosynthetic bacteria (PSB) showed no notable effects. Transplanting at 16 days after sowing produced the tallest plants, with an average height of 10.59 cm, significantly higher than those transplanted at 10 and 13 days. In contrast, the highest number of leaves was recorded in plants transplanted at 10 days, averaging 12.08 leaves, which was significantly greater than those transplanted at 13 and 16 days. Leaf width did not differ significantly across transplanting ages, although 10-day transplants showed slightly wider leaves. PSB application at different times (0, 3, 7, and 10 days after planting) did not significantly impact any measured growth parameters, including plant height, leaf width, or number of leaves. Similarly, no significant interaction effects were found between transplanting age and PSB application timing, suggesting that transplanting age alone independently determined red lettuce performance. These findings highlight that optimal transplanting age is a critical factor for enhancing either plant height or leaf production in hydroponic red lettuce, while PSB application under the tested conditions did not contribute to measurable improvements. The results suggest prioritizing transplanting schedule over microbial enhancement for efficient and sustainable hydroponic cultivation of red lettuce.

1. Introduction

Red lettuce (*Lactuca sativa* var. *Crispa*) is widely appreciated in both commercial and home settings due to its vibrant color, high nutritional value, and adaptability to various growing conditions. As a valuable leafy vegetable, red lettuce offers essential nutrients like vitamins A, C, and K, alongside iron and beneficial antioxidants such as anthocyanins, which contribute to human health by combating free radicals [5]. Driven by an increasing consumer demand for fresh, nutrient-dense produce, hydroponic systems have become popular for cultivating leafy vegetables like red lettuce. Hydroponics, a soil-free plant production system, allows for precise control over nutrient delivery, water usage, and environmental conditions, making it ideal for producing high-yield, high-quality vegetables [13]. Among hydroponic methods, the Nutrient Film Technique (NFT) is one of the most

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efficient due to its continuous flow of nutrient solution, which provides an optimal balance of water, oxygen, and nutrients to plant roots [3]. However, maximizing growth and yield in hydroponic systems depends on various factors, including transplanting age and the application of biological growth enhancers such as photosynthetic bacteria.

Transplanting age plays a critical role in the establishment and overall growth of hydroponic crops. Plants transplanted at the right developmental stage are more likely to establish quickly, ensuring robust root development and faster growth. In lettuce cultivation, transplanting at an inappropriate age can lead to stress, reduced nutrient uptake, and stunted growth. Studies have shown that optimal transplanting age varies depending on the crop species, environmental conditions, and cultivation system. For example, research by [9] demonstrated that younger seedlings of leafy vegetables like lettuce tend to adapt more quickly to hydroponic conditions, leading to improved biomass accumulation and yield. However, there is still limited understanding of the specific effects of transplanting age on red lettuce, particularly in hydroponic systems, making this an important area for further investigation.

Photosynthetic bacteria (PSB) have recently gained attention for their potential to enhance plant growth and health. PSB, including species from the genera Rhodopseudomonas and Rhodospirillum, are microorganisms capable of converting solar energy into chemical energy through photosynthesis, similar to plants [17]. These bacteria play a vital role in promoting plant growth by enhancing nutrient availability, fixing nitrogen, and improving root health. When introduced into hydroponic systems, PSB have been reported to improve nutrient absorption, reduce disease incidence, and increase biomass in a variety of crops. According to Baba et al [4], photosynthetic bacteria (*Synechococcus* sp) are one type of photosynthetic bacteria from the cyanobacteria group, that can penetrate plant leaf tissue and carry out photosynthesis while being able to bind free nitrogen in the atmosphere.

Research into the application of PSB in hydroponics is still in its infancy, but early studies suggest promising results. For example, a study by Lee et al [10] demonstrated that PSB applications improved the growth rate and nutrient uptake in leafy vegetables such as spinach and lettuce. Furthermore, it has been suggested that PSB can mitigate some of the stresses associated with hydroponic cultivation, such as limited root oxygenation and nutrient imbalances [5]. Despite these advances, few studies have examined the specific effects of PSB on red lettuce under hydroponic conditions.

This study aimed to evaluate the combined effects of transplanting age and the application of photosynthetic bacteria on the growth performance and yield of red lettuce cultivated in a hydroponic system. By exploring these factors, this research contributes to optimizing red lettuce production practices, potentially improving quality in commercial hydroponic setups. The study assesses key growth to determine the most effective transplanting age and PSB application rate for red lettuce under hydroponic conditions. By understanding how transplanting age and biological enhancers like PSB influence growth, this research aims to provide practical recommendations for red lettuce producers using hydroponic systems, thereby promoting more efficient and sustainable cultivation methods.

2. Materials and methods

2.1 *Experimental Site and Conditions*

The study was conducted from April to June 2024 at the ARD Farm Nogosari Garden, Boyolali, located at an altitude of approximately 152 meters above sea level. The experiment was set up in a semi-controlled greenhouse environment, which allowed for partial control over temperature and humidity. Average daily temperatures ranged between 25°C and 30°C, and humidity levels were monitored to maintain relative stability at around 60-70%. Light intensity in the greenhouse was supplemented with LED grow lights when natural light was insufficient, ensuring an average photoperiod of 12 hours per day, as recommended for optimal lettuce growth.

2.2 Hydroponic System Setup

The study utilized a Nutrient Film Technique (NFT) hydroponic system. The NFT system was installed on an 8-meter-long table with a width of 1.5 meters and a slope of 3% along the length to facilitate nutrient flow. Prior to the experiment, the NFT table was thoroughly cleaned with tap water to eliminate any residues. For nutrient delivery, an A/B mixture fertilizer solution was prepared, consisting of equal parts of macronutrients and micronutrients essential for lettuce growth [2]. The nutrient solution was continuously circulated through the NFT channels using a submersible water pump, which maintained a steady flow to ensure that each plant received an adequate and consistent supply of nutrients.

To achieve optimal nutrient conditions, the pH and Total Dissolved Solids (TDS) of the solution were carefully monitored and adjusted daily. The pH was maintained between 6.2 and 7.0, while the TDS was kept within a range of 560 to 840 ppm. These parameters were checked using a digital pH meter and a TDS meter, with adjustments made as necessary to stay within the ideal ranges for lettuce.

2.3 Preparation and Transplanting of Seedlings

Red lettuce seeds (*Lactuca sativa* var. *Crispa*) were purchased from a certified supplier to ensure quality and uniformity. The seeds were germinated in rock wool cubes measuring 1.5 cm x 1.5 cm, with two seeds sown per cube to maximize germination rates. Rock wool cubes were first soaked in pH-balanced water to provide an ideal environment for seed germination. After sowing, the trays of rock wool cubes were placed in a shaded area within the greenhouse and misted regularly with distilled water to maintain adequate moisture.

Seedlings were monitored daily, and only those showing uniform growth were selected for transplantation. Three transplanting ages were tested: 10, 13, and 16 days after sowing (DAS). At each transplanting stage, seedlings were carefully removed from the rock wool cubes and transferred to the NFT system's planting holes. Seedlings were spaced evenly to prevent competition and ensure equal access to light and nutrients. Transplanting was conducted in the morning to minimize transplanting shock and ensure a smooth adaptation to the hydroponic environment.

2.4 Application of Photosynthetic Bacteria

Photosynthetic bacteria (PSB) from the cyanobacteria group, specifically *Synechococcus* sp., were used for the foliar application. A PSB solution was prepared at a concentration of 2 mL/L. PSB was applied as a foliar spray at four different intervals post-transplant: 0, 3, 7, and 10 days after planting (DAP). Each foliar application was administered in the early morning to minimize evaporation and ensure effective absorption.

During the application, plastic sheets were used to cover the surrounding plants to prevent cross-contamination and ensure that only the designated plants received the PSB treatment. A handheld sprayer was used to deliver the solution evenly across the leaf surface, ensuring thorough coverage without runoff.

2.5 Growth Parameters

To evaluate the effects of transplanting age and PSB application, three key growth parameters were measured after 28 days of growth.

Plant Height
Measured from the root neck to the tip of the tallest leaf using a measuring tape. Measurements were recorded in centimeters to the nearest millimeter.

Leaf Width
Determined by measuring the width of the third leaf from the base of the plant (a fully expanded leaf) using a measuring tape. Leaf width was recorded in centimeters.

Number of Leaves
The number of fully developed leaves was counted for each plant, including only leaves that had reached a sufficient size to be classified as mature.

Each parameter was recorded for all plants within each treatment group, and averages were calculated to assess differences between groups.

2.6 Experimental Design and Statistical Analysis

The experiment was conducted using a completely randomized design (CRD) with a two-factor factorial arrangement. The two main factors were transplanting age (three levels: 10, 13, and 16 DAS) and the timing of PSB application (four levels: 0, 3, 7, and 10 DAP). Each treatment combination was replicated five times, with each replicate containing five plants, resulting in a total of 15 plants per treatment.

Data were analyzed using Analysis of Variance (ANOVA) to evaluate the main effects of transplanting age and PSB application, as well as their interaction effects on the growth parameters. Post hoc comparisons of means were performed using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$ to identify specific differences between treatment levels. All statistical analyses were conducted using the statistical software and results were presented as means ± standard errors.

3. Results

3.1 Effect of Transplanting Age on Growth Parameters

3.1.1 Plant Height

ANOVA revealed a statistically significant effect of transplanting age on plant height (Figure 1). Plants transplanted at 16 days after transplanting (DAT) achieved the highest average height, followed by those transplanted at 13 DAT, and 10 DAT. Post hoc comparisons using the Least Significant Difference (LSD) test showed that the height difference between 16 DAT and 10 DAT was statistically significant. However, no significant height difference was observed between the 16 DAT and 13 DAT groups, indicating a diminishing effect of transplanting age on height as the seedlings mature.

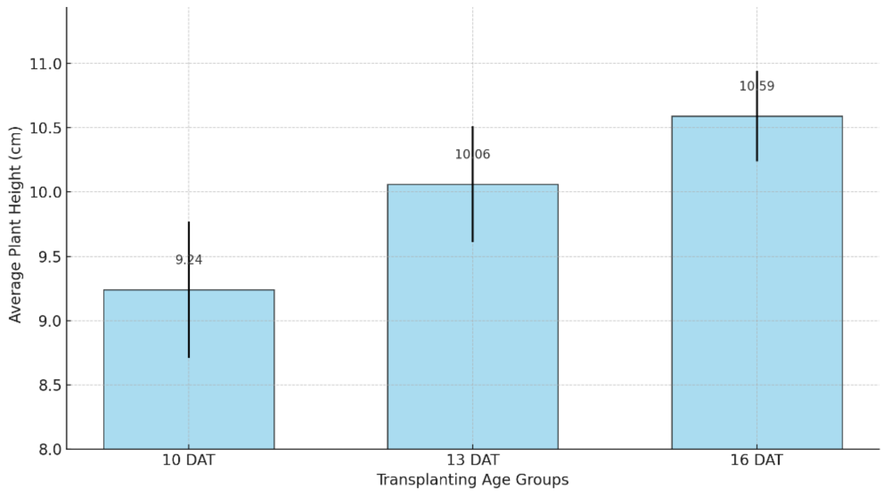

Fig. 1. Effect of transplanting age on the plant height of red lettuce

3.1.2 Leaf Width

Figure 2 shows transplanting age showed no statistically significant effect on leaf width. Although plants transplanted at 10 DAT exhibited slightly wider leaves compared to those at 13 DAT and 16 DAT, these differences did not reach statistical significance. The absence of significant differences across groups suggests that leaf width may be less sensitive to transplanting age than other parameters, such as plant height.

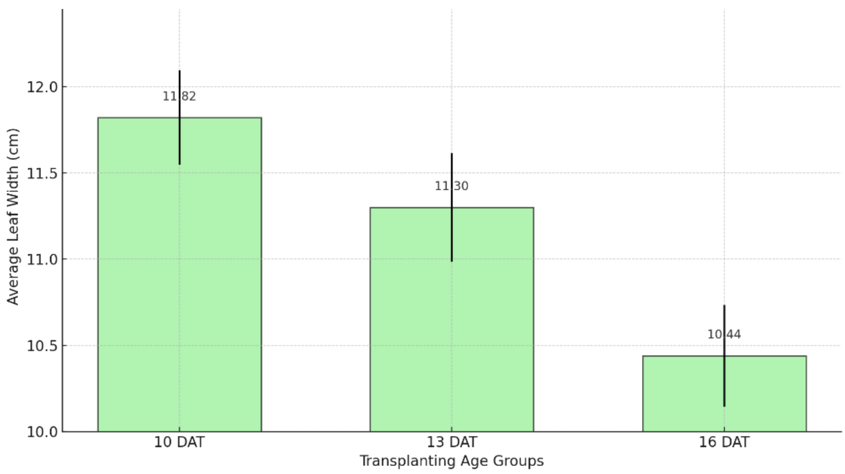

Fig. 2. Effect of transplanting age on the leaf width of red lettuce

3.1.3 Number of Leaves

A significant effect of transplanting age on the number of leaves was observed in Figure 3. Plants transplanted at 10 DAT had the highest leaf count, followed by those at 13 DAT, and 16 DAT. Pairwise comparisons revealed significant differences between the 10 DAT and 16 DAT groups, and between the 10 DAT and 13 DAT groups. These findings indicate that early transplanting at 10 DAT encourages greater leaf production, a critical attribute for leafy crops like lettuce, where leaf quantity is directly related to yield.

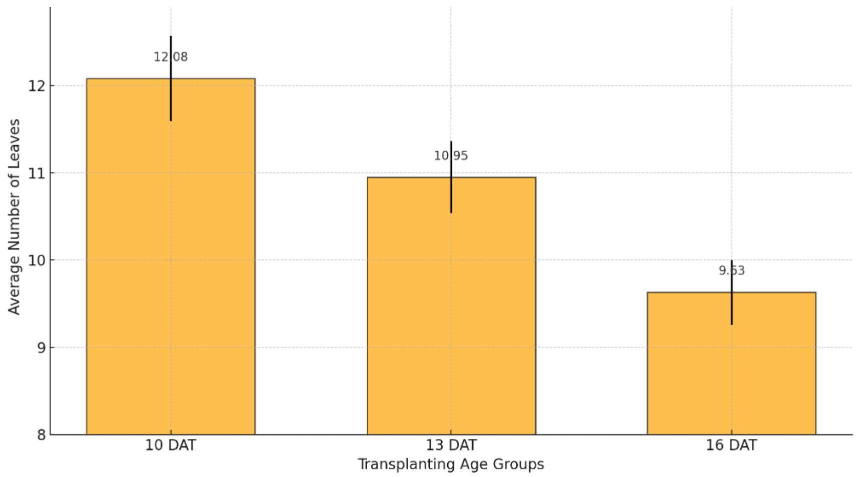

Fig. 3. Effect of transplanting age on the leaf count of red lettuce

3.2 Effect of Photosynthetic Bacteria Application on Growth Parameters
3.2.1 Plant Height

Figure 4 depicted the timing of photosynthetic bacteria (PSB) application did not produce a statistically significant effect on plant height. Across all application timings (0, 3, 7, and 10 days after planting, DAP), plant heights were similar, with means ranging narrowly from 9.64 cm to 10.10 cm. This indicates that under the hydroponic conditions of this study, PSB application timing did not enhance plant height.

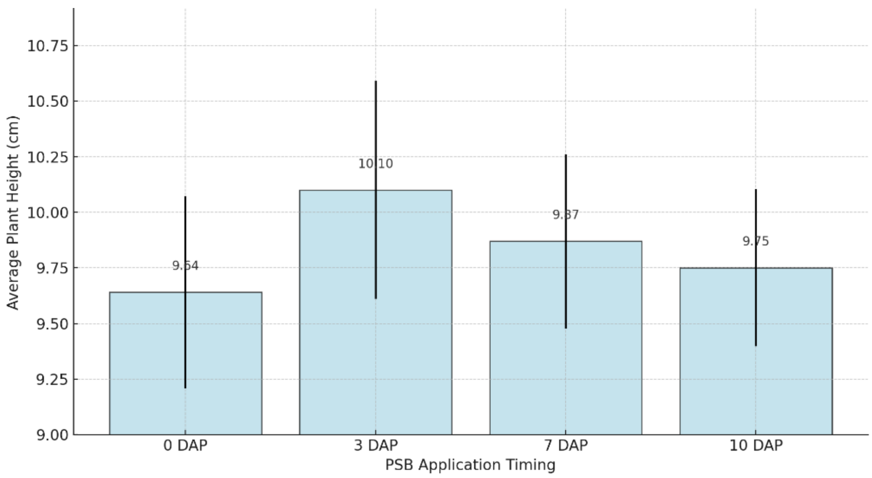

Fig. 4. Effect of PSB application timing on the plant height of red lettuce

3.2.2 Leaf Width

No significant effect of PSB application timing was found on leaf width (Figure 5). Leaf widths across all PSB application timings ranged from 11.00 cm to 11.32 cm, showing minimal variation. This suggests that PSB application does not impact leaf expansion in hydroponic red lettuce, perhaps due to the controlled nutrient environment already optimized for growth.

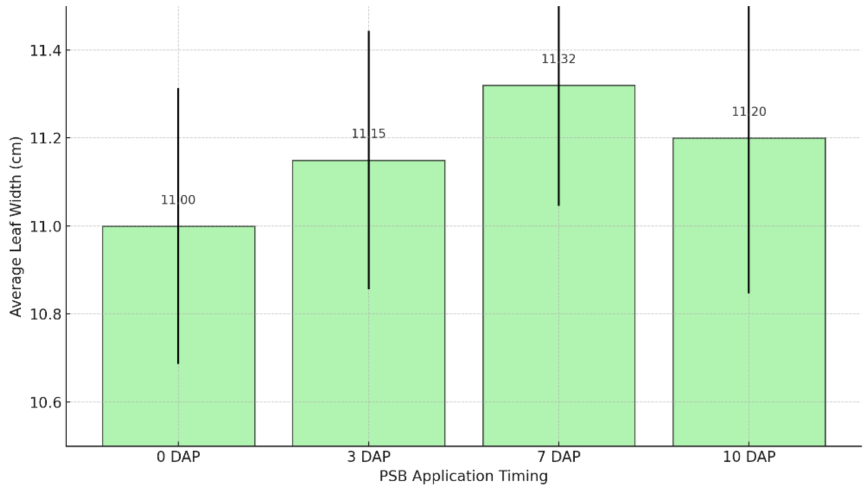

Fig. 5. Effect of PSB application timing on the leaf width of red lettuce

3.2.3 Number of Leaves

The timing of PSB application did not significantly influence the number of leaves (Figure 6). Leaf counts across different PSB application timings ranged from 10.06 to 11.50, showing no significant enhancement from bacterial application. This outcome suggests that PSB may have a limited role in promoting leaf production under the nutrient-rich conditions of the hydroponic system used in this study.

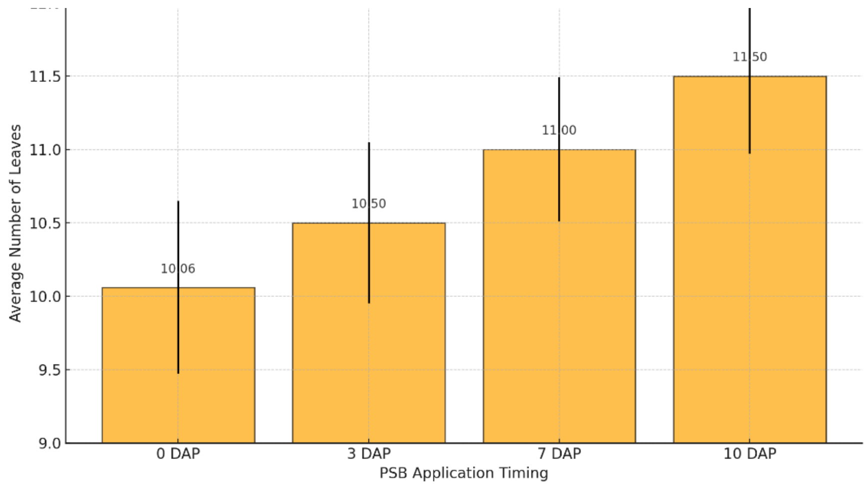

Fig. 6. Effect of PSB application timing on the number of leaves of red lettuce

3.3 Interaction Effects between Transplanting Age and Photosynthetic Bacteria Application

Interaction effects between transplanting age and PSB application timing were analyzed to determine if these factors combined to produce any synergistic effects on plant growth. No significant interaction was found between transplanting age and PSB application timing for plant height (Figure 7). This indicates that while transplanting age independently influences plant height, the addition of PSB does not enhance or modify this effect. Similarly, no significant interaction effect was detected for leaf width, confirming that the influence of transplanting age on leaf morphology operates independently of PSB application timing. In terms of number of leaves, no significant interaction effect was observed. The data suggest that transplanting age significantly affects leaf production, but the timing of PSB application does not enhance or modify this effect.

Fig. 7. Interaction effects between transplanting age and PSB application timing on the plant growth of red lettuce

4. Discussion

The results of this study on the influence of transplanting age and the application of photosynthetic bacteria on red lettuce growth using a hydroponic system reveal key insights into the optimal transplanting time and the limited role of photosynthetic bacteria in enhancing growth parameters such as plant height, leaf width, and the number of leaves. The findings are crucial for improving hydroponic cultivation practices for lettuce.

4.1 Transplanting Age Effects on Plant Height, Leaf Width and Number of Leaves

In this study, transplanting age significantly affected plant height, with the tallest plants observed at 16 days after transplanting (DAT). The differences between transplanting at 10, 13, and 16 DAT were significant, where the plants transplanted at 16 DAT reached a height of 10.59 cm, whereas those transplanted earlier (10 DAT) had a shorter height (9.24 cm). This suggests that red lettuce benefits from a longer vegetative growth period before being transplanted, allowing for better establishment and higher plant height.

Other studies have also demonstrated that extending the time before transplanting can lead to more robust growth, as the seedlings have more time to develop a strong root system and adjust to the hydroponic environment (Jeong et al., 2024). When seedlings are given more time before transplanting, their root systems have a chance to develop more extensively in the seedling stage [8]. A well-developed root system is crucial for nutrient and water uptake, especially in hydroponic systems where the nutrient solution is the sole source of nourishment. Seedlings with a more developed root network are more efficient at absorbing nutrients from the nutrient solution. A larger root surface area means that the plant can absorb more nutrients and water simultaneously, facilitating better overall growth, as the plant has sufficient resources to support both shoot and leaf expansion. Rajaseger et al [14]stated that in hydroponics, the constant availability of nutrients means that more developed roots can take full advantage of the solution, leading to better growth rates, particularly in later stages like plant height and leaf development. In the present study, transplanting red lettuce at 16 DAT appears to optimize plant height.

Interestingly, transplanting age had no statistically significant effect on leaf width, even though there were slight variations. Leaves were widest at 10 DAT (11.82 cm) and smallest at 16 DAT (10.44 cm). The absence of significant differences suggests that the timing of transplanting does not strongly influence leaf expansion in red lettuce under these conditions. This finding contrasts with some other hydroponic crops where transplanting age has been linked to leaf development, possibly due to differences in species' response to environmental factors.

Transplanting age had a significant effect on the number of leaves, with the highest number recorded for plants transplanted at 10 DAT (12.08 leaves). This may be due to early transplanting giving the plants sufficient time to produce more leaves compared to later transplanting at 16 DAT, where leaf count was lower (9.63 leaves). These results emphasize that while delayed transplanting improves plant height, early transplanting may be more favorable for maximizing leaf count, a key quality parameter for leafy vegetables like lettuce.

In the case of lettuce, a leafy vegetable, the number of leaves is often a key quality parameter, as leaves represent the edible part of the plant. Early transplanting encourages the plant to allocate resources more towards vegetative growth (leaf production) rather than height [12]. When transplanted earlier, plants are smaller and may focus on expanding their leaf area to capture more light. This leads to an increase in the number of leaves, which is a crucial attribute for leafy crops like lettuce. Early transplanting allows the plant to start developing its leaf area sooner, leading to more leaves over the course of its growth cycle. This increase in leaf number enhances the plant’s ability to photosynthesize, contributing to better overall health and productivity [11]. In relation of balancing energy allocation, plants that are transplanted early tend to allocate energy towards expanding their canopy (leaf count) rather than vertical growth [19]. This is because, at the early stages of development, plants prioritize expanding their photosynthetic surface (leaves) to capture more light and produce energy. For lettuce, this means more leaves are developed in response to early transplanting.

4.2 Photosynthetic Bacteria Application Effects

The application of photosynthetic bacteria at different days after planting did not significantly impact any of the growth parameters (plant height, leaf width, and number of leaves) across the four application timings (0, 3, 7, and 10 DAP). This finding indicates that the timing of bacterial application did not provide any added benefit to the overall growth of red lettuce under hydroponic conditions.

The lack of a significant effect on plant height suggests that either the hydroponic system's nutrient delivery was already optimized, or the photosynthetic bacteria did not enhance nutrient uptake or photosynthesis under these conditions. Similarly, no significant improvements in leaf width or leaf count were observed with bacterial application. This contradicts some studies where the use of microbial inoculants has promoted leaf growth by increasing nutrient availability or improving stress tolerance in crops [18]. However, it is possible that the concentration or type of photosynthetic bacteria used in this experiment was not ideal for red lettuce, or the plants were not under conditions that would elicit a beneficial microbial effect.

4.3 Interaction Between Transplanting Age and Photosynthetic Bacteria Application

None of the interactions between transplanting age and photosynthetic bacteria application were significant for plant height, leaf width, or the number of leaves. This suggests that the effect of transplanting age on red lettuce growth operates independently of the timing of bacterial application. In other words, while transplanting age clearly affects plant height and the number of leaves, the addition of photosynthetic bacteria does not enhance or modify these effects.

5. Conclusion

These results highlight the importance of transplanting age in determining key growth outcomes for red lettuce in hydroponic systems. The optimal transplanting age varies depending on the specific growth parameter of interest such as for taller plants, transplanting at 16 DAT is ideal. Meanwhile, for maximizing leaf count, 10 DAT is preferable. The lack of significant effects from photosynthetic bacteria application suggests that, under the conditions of this study, microbial amendments did not substantially improve growth outcomes. This could lead to the conclusion that, for red lettuce under similar hydroponic conditions, optimizing transplanting practices may be more critical than focusing on microbial inoculation. Further research could explore different strains or concentrations of photosynthetic bacteria, as well as their effects under varying environmental conditions such as light intensity or nutrient concentration, which could potentially yield more positive results. Additionally, investigating different transplanting ages beyond the range tested here may provide further insights into the optimal timing for hydroponic systems.

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