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Comparative Analysis of Growth Dynamics and Biochemical Composition of Lettuce Cultivars in Nutrient Film Technique and Ebb and Flow Hydroponic Systems

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Abstract
In response to the growing need for sustainable and resource-efficient agricultural systems, hydroponics has emerged as a viable approach for high-yield crop production under controlled environments. This study assessed the growth performance and biochemical attributes of five lettuce cultivars—Alanis, Aleppo, Carmesi, Saturday, and Kiribati—cultivated under two hydroponic systems: Nutrient Film Technique (NFT) and Ebb and Flow (E&F). Key parameters evaluated included leaf number at multiple stages (7, 14, 20, and 25 days after planting), plant diameter, fresh biomass, nitrate content, and carbohydrate concentration. Two-way ANOVA revealed that hydroponic system type significantly influenced early leaf development ($p < 0.001$), plant diameter, and carbohydrate content, while cultivar effects were significant only for leaf number at 20 days and nitrate content. Notably, Alanis exhibited superior growth performance, with the highest leaf count (21), plant diameter (21.66 cm), and biomass (281.17 g), along with elevated nitrate levels (1055 mg/kg). Kiribati also performed well in terms of biomass (235.50 g) and nitrate accumulation (961.5 mg/kg). While Carmesi showed moderate vegetative growth (145.50 g biomass), it excelled in nutritional quality, displaying the highest average carbohydrate content (4.30 g/100 g) and the lowest nitrate concentration (559.5 mg/kg). Overall, the NFT system favored vegetative development across cultivars, particularly for Alanis and Kiribati, whereas Carmesi stood out for its biochemical composition. These findings highlight the critical role of system design and cultivar-specific responses in optimizing hydroponic crop outcomes. The integration of agronomic and nutritional metrics provides a comprehensive framework for informed cultivar selection in controlled-environment agriculture.

Keywords: Growth Dynamics, Biochemical Composition, Lettuce Cultivars

1. Introduction

Lettuce (Lactuca sativa L.) is a cornerstone of global horticulture, valued for its nutritional richness, including vitamins (A, C, K), minerals (e.g., calcium, potassium), and bioactive compounds like phenolic antioxidants. Hydroponic systems, such as Nutrient Film Technique (NFT) and Ebb and Flow (EF), implemented within controlled greenhouse environments, offer sustainable cultivation methods by optimizing water and nutrient use efficiency. Greenhouses provide stable microclimatic conditions—temperature (20–25°C), relative humidity (60–80%), and light intensity (200–400 µmol m^{-2} s^{-1})—that enhance hydroponic performance, shielding lettuce from external climate variability while enabling precise control over growth parameters. The distinct nutrient delivery mechanisms of NFT, with its continuous shallow nutrient flow, and EF, with periodic flooding and draining, differentially influence lettuce growth dynamics (e.g., leaf area, biomass, root morphology) and biochemical composition (e.g., chlorophyll, flavonoids, nutrient content). This greenhouse-based study aims to compare these effects across lettuce cultivars to optimize yield and nutritional quality. In NFT systems, a continuous nutrient film bathes roots, ensuring high oxygen availability and efficient nutrient uptake, which enhances photosynthetic rates and biomass accumulation. Nițu *et al*. (2024) [14] demonstrated in greenhouse trials that elevated root-zone oxygen (8–10 mg L^{-1}) under LED illumination (200–300 µmol m^{-2} s^{-1}) in NFT systems increased lettuce fresh weight by 15–20%, driven by enhanced root respiration and nutrient assimilation supporting cell division and leaf expansion. Conversely, EF systems periodically flood roots, followed by drainage to promote oxygenation, which may enhance root vigor but risks nutrient variability. Fontana *et al*. (2020) [11] reported that greenhouse-based EF systems achieved 54.3% higher water use efficiency (WUE) in closed-loop setups compared to open systems, though periodic flooding can cause transient nutrient imbalances, affecting chlorophyll synthesis and metabolite production.

Cultivar-specific responses amplify these differences. Nițu *et al*. (2025) [15] found in greenhouse experiments that cultivar like 'Crispilla' and 'Gentilina' in NFT systems with oxygen-enriched water (9 mg L^{-1}) and LED lighting (250 µmol m^{-2} s^{-1}, 16 h photoperiod) exhibited a 25% increase in phenolic content, attributed to upregulated phenylpropanoid biosynthesis under high oxygen levels. In EF systems, cultivars like 'Romaine' showed higher biomass due to periodic nutrient surges, as noted by Velazquez-Gonzalez *et al*. (2022) [18], though excessive flooding cycles can stress sensitive cultivars, reducing flavonoid accumulation. Greenhouse conditions, such as stable temperatures and controlled light, further modulate these outcomes. For instance, Sublett *et al*. (2018) [16] reported that an electrical conductivity (EC) of 1.8 dS m^{-1} in greenhouse NFT systems optimized nutrient uptake without biomass reduction, while EF systems required precise cycle timing to prevent nutrient accumulation.

A study reported the urgent need for sustainable soil and water conservation strategies to combat the growing threats posed by climate change. Hafeez *et al*. (2024) [12] highlighted that water conservation strategies in greenhouse hydroponics, including optimized nutrient delivery, are critical under changing climatic conditions, particularly in water-scarce regions. Suleman *et al*. (2025) [17] suggested alternative solutions, use of Biochar to enhance plant nutrition and stress resilience, boosting crop productivity amid changing environmental conditions. Additionally, insights from related greenhouse studies, such as Arshad *et al*. (2024) [10] on cherry tomatoes, suggest that precise nutrient management (e.g., NPK ratios of 150:50:200 mg L^{-1}) in hydroponic systems enhances biochemical quality, a principle applicable to lettuce. These findings underscore the need for tailored system design and cultivar selection to maximize resource efficiency and nutritional output in greenhouse hydroponics.

This greenhouse-based study aims to compare the growth dynamics (e.g., plant height, leaf number, fresh and dry biomass) and biochemical composition (e.g., chlorophyll, phenolic compounds, macro- and micronutrients) of selected lettuce cultivars in NFT and EF hydroponic systems. By elucidating the interplay between system design, cultivar-specific responses, and greenhouse microclimatic conditions, this analysis seeks to provide actionable insights for optimizing hydroponic lettuce production for yield, nutritional quality, and resource efficiency in controlled environment agriculture.

2. Materials and Methods

2.1 Experimental Site and Greenhouse Conditions

The experiments were conducted in controlled greenhouse environments at two facilities: the Hortinvest Research Center Greenhouse at the University of Agronomic Sciences and Veterinary Medicine (USAMV), Bucharest, Romania, for the Nutrient Film Technique (NFT) system, and the Lolita Greenhouses for the Ebb and Flow (EF) system. Both greenhouses maintained stable microclimatic conditions to optimize lettuce growth, with temperatures ranging from 20–25°C, relative humidity of 60–80%, and light intensity of 200–400 µmol m^{-2} s^{-1} provided by natural daylight supplemented with LED lighting (16 h photoperiod). Environmental parameters were monitored and controlled to ensure consistency across the experimental period. Five lettuce (Lactuca sativa L.) cultivars were selected for their diverse growth characteristics: V1 'Alanis', V2 'Aleppo', V3 'Carmesi', V4 'Saturday', and V5 'Kiribati'. Seeds were sourced from certified commercial suppliers and germinated under uniform conditions prior to transplanting into the respective hydroponic systems.

Fig 1:compartments within greenhouse specified for the growth of lettuce

2.2 Hydroponic Systems

2.2.1 Nutrient Film Technique (NFT)

The NFT system was established in a dedicated compartment of the Hortinvest Research Center Greenhouse. Seedlings were transplanted into channels with a continuous flow of nutrient solution (EC: 1.8 dS m^{-1}, pH: 5.5–6.0) containing balanced NPK (150:50:200 mg L^{-1}) and micronutrients (e.g., calcium, magnesium). The nutrient solution was circulated at a flow rate of 1.5 L min^{-1}, ensuring high oxygen availability (8–10 mg L^{-1}) in the root zone, as described by Nițu *et al.* (2024) [14]. The system was designed to maintain consistent nutrient delivery and minimize substrate-related loss.

2.2.2 Ebb and Flow (EF)

The EF system was set up at the Lolita Greenhouses, utilizing trays that were periodically flooded with nutrient solution (EC: 1.8 dS m^{-1}, pH: 5.5–6.0) matching the NFT composition. Flooding cycles occurred every 4 hours for 15 minutes, followed by drainage to promote root oxygenation. The system was configured to optimize water use efficiency while preventing nutrient accumulation, as outlined by Fontana *et al*. (2020) [11].

2.3 Experimental Design

The experiment followed a randomized complete block design with three replicates per cultivar in each hydroponic system. Each replicate consisted of 10 plants, resulting in 30 plants per cultivar per system. Seedlings were transplanted at the two-leaf stage and grown for 25 days under uniform greenhouse conditions. Nutrient solutions were monitored daily and adjusted to maintain consistent EC and pH levels.

2.4 Data Collection

Growth dynamics were assessed by measuring the following parameters at 7, 14, 20, and 25 days after planting (DAP). The total number of fully expanded leaves per plant was counted to evaluate vegetative development. Plant Diameter was measured as the maximum diameter (cm) across the plant to assess vegetative vigor. Fresh biomass (g) was recorded at 25 DAP by harvesting plants, including shoots and roots, and weighing immediately to avoid desiccation.

Measurements were taken using a digital caliper for plant diameter and a precision balance (accuracy ±0.01 g) for biomass. Data were collected from all replicates to ensure statistical robustness.

2.5 Statistical Analysis

Data were analyzed using two-way ANOVA to assess the effects of cultivar and hydroponic system on growth parameters and biochemical components. Significant differences ($p < 0.05$) were observed in leaf number, plant diameter, and biomass among cultivars and between systems. Post-hoc comparisons were performed using Tukey's HSD test. All statistical analyses were conducted using Jamovi (version 2.5.5, The Jamovi Project, 2021).

3. Results

Five lettuce varieties (Alanis, Aleppo, Carmesi, Saturday, and Kiribati) were assessed over a 25-day growth period under two hydroponic systems—Nutrient Film Technique (NFT) and Ebb and Flow—to evaluate their growth and biochemical characteristics (Fig 5.). All varieties exhibited progressive leaf development, with distinct genotypic and system-related differences emerging over time (Table 1). However, the magnitude and rate of leaf emergence varied significantly among the genotypes. A two-way ANOVA was conducted to examine the effects of Variety and Hydroponic System.

At 7 DAP, all varieties exhibited early leaf development, with significantly higher leaf counts in the NFT system compared to Ebb and Flow ($p < 0.001$). There was no significant difference among varieties ($p = 1.00$), indicating uniform growth at this stage. By 14 DAP, divergence in performance began to emerge. Kiribati and Alanis showed the highest leaf numbers (10.99 and 10.16, respectively), while Aleppo had the lowest (7.50). Despite these visible differences, neither the Variety ($p = 0.963$) nor the System effect ($p = 0.259$) reached statistical significance (Fig 2 & 3).

Table 1: Leaf Growth Dynamics (Mean ± SD) of Five Lettuce Varieties (Alanis, Aleppo, Carmesi, Saturday, and Kiribati) Cultivated under Two Hydroponic Systems—Nutrient Film Technique (NFT) and Ebb and Flow (E&F)

Verities	No. of leaves after 7 days	No. of leave after 14 days	No. of leave after 20 days	No. of leave after 25 days	Total number of leaves
Alanis	4.16	10.16	18.44	20.995	3.305
Aleppo	3.83	7.495	9.83	16.995	3.34
Carmesi	3.82	7.995	13.5	18.66	3.66
Saturday	4.16	8.83	11.83	15.33	3.31
Kiribati	4.16	10.995	14.995	16.995	3.32
Variety	1.00 ns	0.963 ns	0.036 *	0.068 ns	0.481ns
Hydroponic system	<.001***	0.259 ns	0.174 ns	0.025 ns	0.952 ns

Here p <0.05……*,0.01…**, 0.001 …***ns= non-Significancelevel.

At 20 DAP, differences among varieties became statistically significant ($p = 0.036$). Alanis continued to lead (18.44 leaves), followed by Kiribati (14.99), while Aleppo remained the lowest performer (9.83). The hydroponic system did not significantly influence leaf count at this stage ($p = 0.174$). By 25 DAP, Alanis maintained the highest leaf number (20.99), with Carmesi (18.66) and Kiribati (16.99) following. Although the effect of Variety ($p = 0.068$) and System ($p = 0.025$) were not statistically significant, the trends suggest that both genotype and system influenced final leaf development. A variable reported as "Total Number of Leaves," ranging from 3.305 to 3.66 across varieties, appeared inconsistent with cumulative leaf count data. For instance, Alanis reached 20.99 leaves at 25 DAP, while its total leaf number was recorded as 3.305. This discrepancy suggests a potential labeling or measurement error. As a result, no significant differences were observed for this variable (Variety: $p = 0.481$; System: $p = 0.952$).

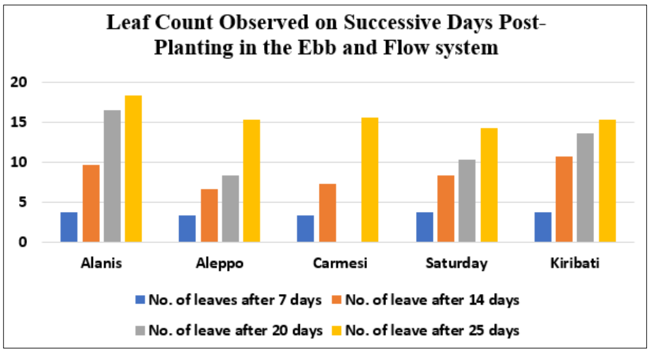

Fig 2: Number of leaves of Five Lettuce Varieties (Alanis, Aleppo, Carmesi, Saturday, and Kiribati) Cultivated under Hydroponic Systems—Nutrient Film Technique (NFT)

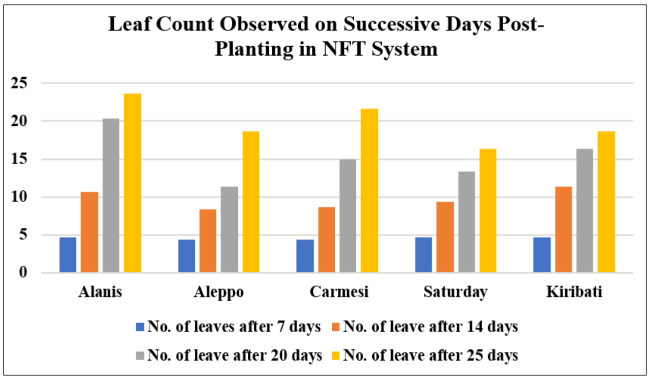

Fig 3: Number of leaves of Five Lettuce Varieties (Alanis, Aleppo, Carmesi, Saturday, and Kiribati) Cultivated under Ebb and Flow (E&F)

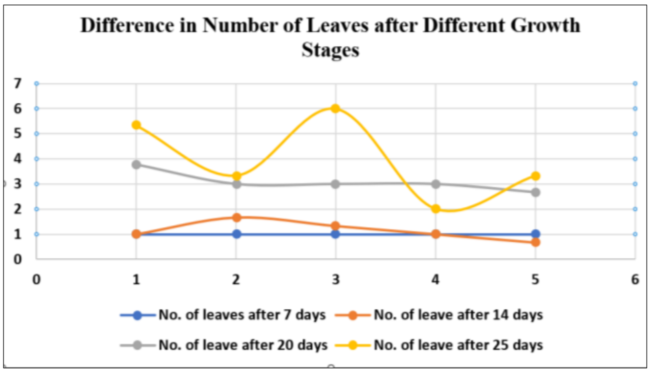

Fig 4: Difference in number of leaves observed on successive days post-planting in NFT System

Fig 5: Different lettuce varieties at the mature growth stage, exhibiting fully developed leaves

All verities appeared with distinct morphological behavior in response to applied hydroponic systems (Fig 6). Plant diameter, a direct measure of canopy spread and vegetative vigor, is a useful indicator of overall plant health and growth performance (Fig 6). All varieties exhibited greater canopy expansion in the NFT system compared to Ebb and Flow, which may be attributed to the continuous and more stable nutrient and oxygen availability in NFT (Table 2). The greatest relative difference in plant diameter was observed in Alanis (a 10 cm reduction in Ebb and Flow), indicating its strong preference for the NFT environment. Aleppo showed the smallest difference between systems, suggesting a more stable but modest performance across varying hydroponic conditions. Plant diameter has also showed a significant effect for both System ($p = 0.003$) and Variety ($p < 0.001$), with larger diameters recorded in the NFT system. Alanis exhibited the greatest diameter (21.66 cm), whereas Aleppo had the smallest (16.33 cm). Despite notable numeric differences in plant mass, with higher values observed in NFT (e.g., Alanis: 281.17 g; Saturday: 285.99 g), neither Variety ($p = 0.816$) nor System ($p = 0.184$) had a significant effect (Fig 7). Nitrate content varied significantly among varieties ($p = 0.002$), with Alanis (1055) and Kiribati (961.5) accumulating the highest levels, but the hydroponic system had no effect ($p = 0.972$) (Fig 8.). In contrast, carbohydrate content was significantly higher in the NFT system (4.386) than in Ebb and Flow (3.512) ($p = 0.004$), while no significant differences were observed among varieties ($p = 0.785$) (Fig 9.)

Table 2: Plant Diameter, plant mass, and chemical components dynamics (Mean ± SD) of Five Lettuce Varieties (Alanis, Aleppo, Carmesi, Saturday, and Kiribati) Cultivated under Two Hydroponic Systems—Nutrient Film Technique (NFT) and Ebb and Flow (E&F)

Verities	Plant Diameter (cm)	Plant mass(g)	Nitrate Contents (mg/kg)	Carbohydrate Contents (mg/kg)
Alanis	21.66	281.165	1055	4.07
Aleppo	16.33	142.995	819	4.03
Carmesi	16.665	145.495	559.5	4.295
Saturday	19.83	285.995	905	3.48
Kiribati	19.995	235.495	961.5	3.87
Variety	< .001***	0.816 ns	0.002**	0.785 ns
Hydroponic system	0.003 **	0.184 ns	0.972 ns	0.004 **

Here p <0.05……*,0.01…**, 0.001 …***ns= non-Significance level

Fig 6: Different morphological traits of lettuce varieties observed in the study area

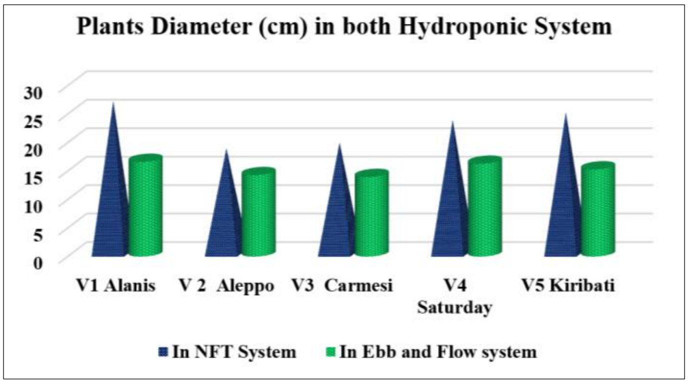

Fig 6: Plants Diameter (cm) in both hydroponic system

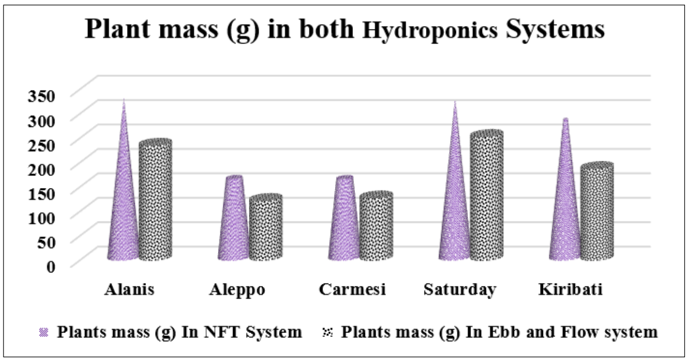

Fig 7: Plant mass (g) in both hydroponics systems

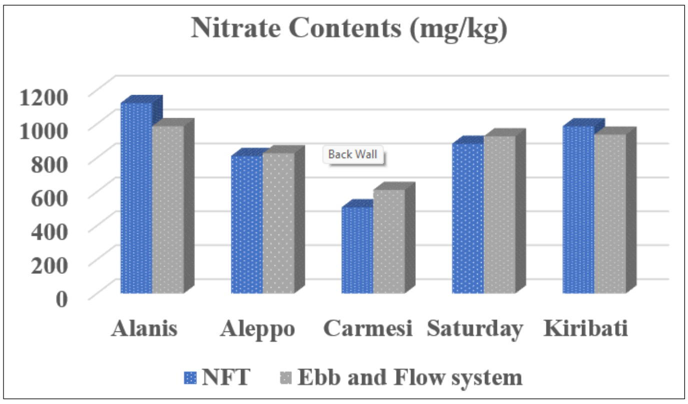

Fig 8: Nitrate Contents (mg/kg)

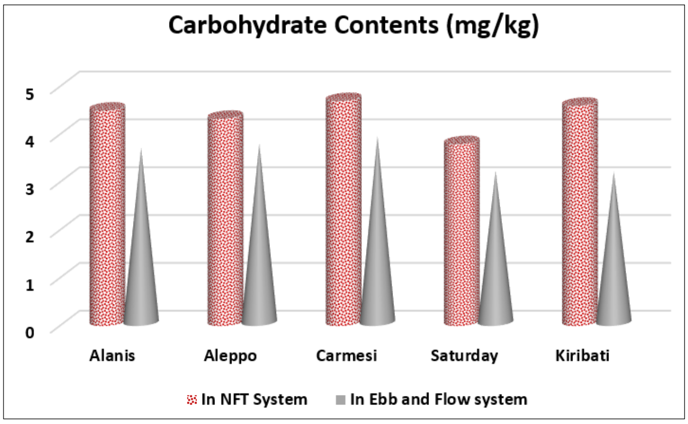

Fig 9: Carbohydrate contents (mg/kg)

4. Discussion

The investigation into two hydroponic systems—Nutrient Film Technique (NFT) and Ebb and Flow (E&F)—and their effects on five lettuce varieties (Alanis, Kiribati, Aleppo, Saturday, and one unnamed variety) reveals distinct influences on plant growth and biochemical properties, driven climatic conditions and underlying biological processes. Different greenhouse microclimate significantly influences vegetation growth in the growing compartment (Arshad *et al*., 2023; Jerca *et al*., 2024) [1, 13]. It has also been reported that different types of fertilizers can also affect the germination rate and early growth performance of lettuce seedlings, aiming to identify which fertilizers promote optimal development during the early stages of cultivation (Chan *et al*., 2023) [3]. Hydroponic systems differ in nutrient delivery and environmental conditions, while the lettuce varieties exhibit genetic variations that shape their growth responses. The NFT system promotes superior leaf development in the early growth phase (around 7 days), likely due to its continuous nutrient flow and high oxygenation. In NFT, a thin stream of nutrient solution flows over the roots, ensuring consistent nutrient availability and oxygen exposure, which stimulate cell division and expansion in leaf primordia (Jones, 2014) [5]. This enhances metabolic processes like respiration and nutrient assimilation, critical for early vegetative growth (Marschner, 2012) [6]. In contrast, the E&F system, which periodically floods the root zone, shows reduced leaf counts, possibly due to intermittent nutrient delivery or lower oxygen availability during non-flood periods, limiting root respiration and energy allocation to leaf formation.

The lettuce varieties display significant genetic differences in growth potential, particularly evident by 20 days. Alanis outperforms other varieties in leaf production, reflecting genotypic advantages in photosynthetic efficiency or resource allocation. Genetic variation influences the expression of genes controlling meristem activity and leaf initiation, as seen in plant physiology studies (Marschner, 2012: Arshad, A. 2021) [6, 2]. For instance, Alanis may upregulate genes involved in cell cycle progression, leading to faster leaf development. By 25 days, the influence of variety diminishes, suggesting that environmental factors from the hydroponic systems may equalize growth as plants acclimate. This acclimation could involve feedback mechanisms, such as nutrient saturation or hormonal regulation, stabilizing growth rates across varieties (Resh, 2022) [9]. The NFT system supports greater plant diameter compared to E&F, likely due to optimized nutrient and water delivery. Larger diameters result from enhanced cell expansion and turgor pressure, driven by efficient nutrient uptake in NFT's continuous flow environment. This system likely supports higher photosynthetic rates, increasing carbohydrate availability for structural growth (Jones, 2014) [5]. The E&F system's lower performance may stem from periodic nutrient exposure, which could disrupt consistent water and nutrient uptake, constraining cell wall synthesis and expansion.

Nitrate content varies significantly among varieties, with Alanis and Kiribati accumulating higher levels. This reflects genetic control over nitrate reductase activity, an enzyme critical for nitrogen assimilation (Hawkesford & Barraclough, 2011). Higher nitrate levels in these varieties may enhance amino acid synthesis, supporting protein production and growth. Carbohydrate content, however, is influenced more by the hydroponic system than by variety. The NFT system's higher carbohydrate levels suggest improved photosynthetic efficiency, possibly due to better nutrient balance or root oxygenation, which boosts carbon fixation and sugar synthesis (Resh, 2022) [9]. The E&F system's periodic flooding may reduce photosynthetic efficiency by temporarily limiting root activity. Future studies should incorporate multiple replicates and consider time as a factor to capture temporal dynamics in growth and biochemical traits. Additionally, standardizing metrics like total leaf counts is critical to avoid inconsistencies that obscure interpretation. The NFT system enhances early leaf development, plant diameter, and carbohydrate accumulation, likely through continuous nutrient delivery and high oxygenation, while varieties like Alanis and Kiribati excel in leaf production and nitrate uptake due to genetic advantages. These findings align with research on hydroponic optimization and varietal differences (Jones, 2014; Marschner, 2012) [5, 6]. Understanding the interplay of environmental and genetic factors in NFT and E&F systems can guide tailored cultivation strategies to maximize yield and quality in lettuce production.

5. Conclusion

The results highlight significant genotypic variability in response to the NFT system. Varieties such as Alanis and Kiribati exhibit promising potential for rapid vegetative development, which is critical for optimizing growth cycles in controlled-environment agriculture. These findings contribute valuable insight for varietal selection in hydroponic production systems, where early leaf formation is a key determinant of biomass accumulation and yield potential. The data confirm notable genotypic variability in response to the Ebb and Flow hydroponic system. Alanis (V1) consistently exhibited the highest number of leaves, indicating superior vegetative vigor and adaptability to periodic nutrient exposure. Kiribati (V5) also demonstrated strong early performance. While Aleppo (V2) started with limited growth, its improvement by 25 DAP suggests potential for longer-term productivity. These findings emphasize the importance of genotype selection in optimizing crop performance under hydroponic systems.

The results demonstrate that the NFT system supported greater vegetative growth in terms of plant diameter across all tested lettuce varieties. Alanis and Kiribati performed exceptionally well in NFT, indicating high adaptability and potential for high-yield production. In contrast, growth was generally reduced in the Ebb and Flow system, though some varieties, such as Saturday, maintained relatively strong performance. These findings underscore the importance of matching cultivar characteristics to specific hydroponic system designs to maximize productivity in controlled-environment agriculture.

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