



TECNOLÓGICO
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Instituto Tecnológico de Pabellón de Arteaga

**“SYNERGISTIC EFFECTS OF NITRATE UNDER LETTUCE ALFALFA
ASSOCIATIONS IN BIOMECHATRONIC NFT VERTICAL FARMING”**

THESIS

To Obtain the Degree of:

Master of Science in Mechatronic Engineering

PRESENTED BY:

I.E. Luis Bryant Diaz Andrade

ADVISORS:

PhD. José Ernesto Olvera González

PhD. Nivia Iracemi Escalante García

PhD. Francesco Orsini

Pabellón de Arteaga, Ags., Friday, May 9th, 2025.



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**INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA
DIVISIÓN DE ESTUDIOS DE POSGRADO E INVESTIGACIÓN
MAESTRÍA EN CIENCIAS EN INGENIERÍA MECATRÓNICA**

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This achievement is ours.



Pabellón de Arteaga, Ags.,

April 3rd, 2025

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ABSTRACT

“SYNERGISTIC EFFECTS OF NITRATE UNDER LETTUCE ALFALFA ASSOCIATIONS IN BIOMECHATRONIC NFT VERTICAL FARMING”

BY: LUIS BRYANT DIAZ ANDRADE

Vertical farming in Nutrient Film Technique (NFT) type hydroponic systems represents a promising strategy for intensive and sustainable food production in urban environments. However, the efficient management of nitrogen, particularly in the form of nitrate (NO_3^-), remains a critical challenge from both physiological and energetic perspectives. This research evaluated the effect of different nitrate concentrations (0, 33, 66, 66, 100, and 133 % of the standard) on growth, photosynthetic efficiency, nutrient uptake, and energy performance of *Lactuca sativa* var. *longifolia*, grown in association with *Medicago sativa* within an automated NFT system.

A controlled biomechatronic environment was designed that integrated environmental sensors, open controllers (ESP32 and Raspberry Pi), spectrum-optimized LED lamps (80W20B), CO₂ generators and real-time monitoring platforms (Grafana). In addition, a physiological measurement system based on photosystem II ($\phi PSII$) was implemented using a JUNIOR-PAM fluorimeter. Variables analyzed included fresh and dry biomass, chlorophyll content (*SPAD*), light use efficiency (*LUE*), energy efficiency (*EUE*), macro- and micronutrient concentration, and photosynthetic efficiency parameters (F_v/F_m , $Y(II)$, NPQ).

Results indicate that the 66% nitrate treatment presented the best balance between yield, energy efficiency and nutrient quality, reducing foliar nitrate accumulation by up to 29% without compromising biomass. The symbiotic association with *M. sativa* favored root pH stability, reduced electrical conductivity, increased the availability of micronutrients such as Zn and Mn, and improved photosynthetic resilience under light stress. These findings demonstrate that the combination of agroecological strategies and biomechatronic tools can optimize physiological and energetic efficiency in NFT systems, thus contributing to the development of a more sustainable, automated and consumer-safe vertical agriculture.

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NOMENCLATURE

Symbol	Description
NFT	Nutrient Film Technique
VF	Vertical Farming
LS	<i>Lactuca sativa</i>
MS	<i>Medicago sativa</i>
NO_3^-	Nitrate ion
pH	Hydrogen potential
EC	Electrical Conductivity (dS m^{-1})
PPFD	Photosynthetic Photon Flux Density ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
SPAD	Relative chlorophyll content index (SPAD units)
$\Phi\text{PSII (Y(II))}$	Effective quantum yield of Photosystem II
F_v/F_m	Maximum quantum yield of Photosystem II
NPQ	Non-photochemical quenching
LUE	Light Use Efficiency ($\text{g DW mol}^{-1} \text{photons}$)
EUE	Energy Use Efficiency (g FW kWh^{-1})
WUE	Water Use Efficiency ($\text{g DW L}^{-1} \text{water}$)

I. INTRODUCTION

The increasing global population, accelerated urbanization, and climate change have created unprecedented pressure on traditional agriculture. As a response, Controlled Environment Agriculture (CEA) has emerged as a promising solution for sustainable and intensive food production in urban areas. CEA systems, particularly vertical farming (VF), allow for year-round crop production by precisely regulating environmental variables such as temperature, humidity, CO₂ concentration, and light intensity. These systems are especially efficient when combined with hydroponic technologies like the Nutrient Film Technique (NFT), which provides a continuous nutrient solution flow to the root zone of crops like *Lactuca sativa* (lettuce).

Among the various nutrients required for plant development, nitrogen plays a central role in photosynthesis, protein synthesis, and biomass accumulation. However, excessive nitrate (NO₃⁻) fertilization can result in physiological imbalances, nitrate accumulation in edible tissues, and environmental concerns. Moreover, in closed-loop NFT systems, maintaining an optimal balance of nitrate is challenging due to continuous recirculation and interactions between nutrients. While previous research has explored nitrate dynamics in static hydroponic systems, limited studies have addressed the effects of fixed nitrate concentration gradients in automated NFT systems, particularly under intercropping conditions. In this context, *Medicago sativa* (alfalfa), a leguminous species capable of biological nitrogen fixation, offers an agroecological alternative to partially reduce synthetic nitrogen inputs. The symbiotic association between *Lactuca sativa* and *M. sativa* has been shown to improve nutrient uptake, stabilize pH, and enhance the antioxidant capacity of lettuce under stress conditions. Integrating this interspecific relationship into a controlled NFT vertical farm can potentially enhance the system's sustainability while maintaining or even improving yield and nutritional quality.

Nevertheless, most existing vertical farming systems focus solely on environmental automation, without considering the physiological status of plants. Recent advances in plant sensing technologies, such as chlorophyll fluorescence measurements, enable real-time monitoring of photosystem II (PSII) activity. Parameters like Fv/Fm (maximum quantum yield), Y(II) (operating efficiency), and NPQ (non-photochemical quenching) are powerful indicators of photosynthetic performance and stress adaptation. These physiological markers can be integrated into biomechatronic systems for adaptive decision-making and improved energy efficiency.

This study aims to evaluate the effects of varying nitrate concentrations and the association with *Medicago sativa* on the growth, nutrient absorption, photosynthetic efficiency, and energy use of *Lactuca sativa* cultivated in an automated NFT vertical farming system. By combining real-time environmental control with physiological monitoring, this research seeks to optimize both crop performance and resource utilization in urban agriculture.

1.1 PROBLEM STATEMENT

Despite the rapid development of vertical farming systems and their growing importance for urban food production, significant challenges remain in optimizing plant nutrition and energy use efficiency. Nitrate (NO_3^-) is the primary form of nitrogen absorbed by plants in hydroponic systems, and while essential for growth, its mismanagement can lead to nutrient imbalances, excessive accumulation in edible tissues, and inefficient resource utilization.

In closed-loop Nutrient Film Technique (NFT) systems, the continuous circulation of nutrient solutions complicates the stabilization of nitrate concentrations, particularly when synthetic fertilizers are the sole source of nitrogen. At the same time, most automated vertical farms focus exclusively on regulating environmental variables such as temperature, humidity, light, and CO_2 without incorporating the actual physiological state of the plant into the decision-making process. This limitation results in a disconnection between the environmental conditions provided and the real-time metabolic needs of the crop.

Furthermore, the potential benefits of intercropping with nitrogen-fixing species such as *Medicago sativa* remain underexplored in soilless systems. While biological nitrogen fixation (BNF) has shown promise in soil-based agriculture, its effects on nutrient availability, plant performance, and photosynthetic efficiency under controlled hydroponic conditions have not been sufficiently studied.

Therefore, there is a critical need to investigate integrated strategies that combine agroecological practices (such as interspecific symbiosis) with advanced monitoring of plant physiological responses. This could lead to more sustainable, efficient, and responsive vertical farming systems capable of meeting urban food demands while minimizing environmental impact.

1.2 OBJECTIVES

1.2.1 General Objective.

Analyze the effects of varying nitrate concentrations and the symbiotic association with *Medicago sativa* on the growth, nutrient uptake, photosynthetic efficiency, and energy use of *Lactuca sativa* cultivated in an automated vertical NFT system with integrated environmental and physiological monitoring.

1.2.2 Specific Objectives

- Quantify the effects of different nitrate concentrations on biomass production and ion accumulation in *Lactuca sativa*.

- Analyze the impact of *Medicago sativa* co-cultivation on root-zone pH , electrical conductivity (EC), and micronutrient uptake in NFT systems.
- Assess the photosynthetic performance of *Lactuca sativa* through chlorophyll fluorescence parameters (F_v/F_m , $Y(II)$, NPQ) under varying nitrate levels and symbiotic conditions.
- Calculate the Light Use Efficiency (LUE) and Energy Use Efficiency (EUE) across treatments, identifying optimal nitrate input levels for maximum energy return.
- Implement and evaluate a biomechatronic system capable of real-time environmental regulation and PSII-based physiological monitoring.

1.3 JUSTIFICATION

Vertical farming systems represent a technological frontier in sustainable food production, especially in response to the challenges posed by urbanization, climate change, and limited arable land. Among these systems, the Nutrient Film Technique (NFT) stands out due to its efficient water and nutrient use, making it particularly suitable for high-value crops such as *Lactuca sativa*. However, optimizing nutrient management—especially nitrogen—remains a critical barrier to achieving maximum productivity and sustainability in these systems.

Nitrate (NO_3^-), the most common form of nitrogen in hydroponics, is essential for plant development but can become problematic when applied excessively or inefficiently. High nitrate levels may lead to environmental concerns and health risks due to nitrate accumulation in edible tissues. Conversely, insufficient nitrogen supply compromises growth and quality. Therefore, establishing optimal nitrate levels is essential for balancing productivity, quality, and resource use efficiency.

In parallel, intercropping with *Medicago sativa*, a leguminous species capable of biological nitrogen fixation, provides a promising agroecological alternative to reduce dependence on synthetic nitrogen fertilizers. While this strategy has been well studied in soil-based systems, its integration into closed-loop hydroponic NFT systems under controlled environmental conditions has been poorly documented. Evaluating how this symbiotic association influences plant physiology, nutrient availability, and metabolic performance in vertical farms can reveal valuable insights for sustainable intensification.

Moreover, most vertical farming systems currently rely on environmental monitoring and automated control based on external variables, without considering the real-time physiological status of the plant. The use of photosystem II ($PSII$) indicators such as F_v/F_m , $Y(II)$ and NPQ offers a unique opportunity to close this gap. By incorporating these parameters into decision-making algorithms, it is possible to improve crop response, reduce waste, and enhance overall system efficiency.

This study is justified by the need to develop vertical farming systems that not only automate environmental control but also adapt to the actual metabolic needs of crops. Through the integration of agroecological strategies, physiological monitoring, and biomechatronic technologies, this research contributes to the advancement of smart, sustainable, and productive urban agriculture systems.

1.4 HYPOTHESIS

The integration of moderate nitrate concentrations with the intercropping of *Medicago sativa* in an automated NFT vertical farming system will significantly improve the growth, ion balance, and photosynthetic performance of *Lactuca sativa* compared to monoculture systems with either low or excessive nitrate inputs. This combined approach will enhance physiological efficiency (F_v/F_m , $Y(II)$, NPQ), chlorophyll content, and energy use metrics (LUE , EUE), while contributing to nutrient stability and reduced nitrate accumulation in edible tissues.

1.5 SCOPE AND LIMITATIONS

This study was conducted in a fully automated NFT vertical farming system designed to simulate controlled environmental farming conditions. The experiment evaluated five nitrate concentration levels and their interactions with *Medicago sativa* in a co-culture setting. In addition, physiological (chlorophyll fluorescence), agronomic (biomass, ion content) and energetic (EUE , LUE) variables were evaluated during a four-week growth cycle. A biomechatronic system incorporating real-time monitoring, sensor networks, and IoT-based data visualization platforms (e.g., Grafana) was implemented for environmental and physiological regulation.

The experiment was conducted on a laboratory experimental scale; the results may not fully reflect results in large-scale commercial systems. Biological nitrogen fixation by *M. sativa* was not directly quantified through nitrogenase activity or isotopic tracing. Environmental variables were controlled to simulate ideal conditions, so external stresses (e.g., pathogens, drought) were not taken into account. The study did not include postharvest analysis (e.g., shelf life, sensory evaluation) or economic evaluation of input-output relationships. Decision making based on PSII parameters was monitored, but not yet coupled to adaptive machine learning control algorithms.

II. THEORETICAL FRAMEWORK

2.1 VERTICAL FARMING AND NFT SYSTEMS

2.1.1 General Conceptualization.

According to (Chinchilla Hernández, 2020), agriculture represents the principal legacy inherited from primitive humans, significantly contributing to the development of humanity. It was created by humans and has accompanied them for millennia.

Vertical farming has emerged as a solution to the growing demand in metropolitan areas for systems that ensure food sovereignty. Its origins date back to the 19th century, when, during the Industrial Revolution in Germany, the need to improve environmental conditions—particularly for children—was recognized, prompting the use of urban land to establish gardens (Guevara Reyes et al., 2024).

VF (Vertical Farming) involves cultivating plants on multiple levels to increase productivity per unit area. It also refers to a highly automated and controlled system that regulates all the variables necessary for optimal plant growth. VF offers countless opportunities to shift from genetic modification to environmental modification, enabling the production of high-quality, consistent crops regardless of weather, soil conditions, or climate change.

According to (Birkby, 2016) vertical farms vary widely in structure and scale, ranging from basic two-level or wall-mounted systems to complex multi-story warehouse installations. However, they all share a core characteristic: the use of one of three soilless growing systems—hydroponics, aeroponics, or aquaponics.

(Kabir et al., 2023) state that vertical farming has emerged as a promising solution to address increasing food demand, the rising impact of urbanization, and limited natural resources, while also ensuring sustainable urban agriculture throughout the year.

(Van Gerrewey et al., 2022) note that vertical farms can be distinguished by their size, type, and intended use. First, there are Plant Factories with Artificial Lighting (PFALs), which are large-scale industrial facilities specifically designed for vertical crop production. Then there are Container Farms, which are modular vertical systems housed within shipping containers—highly mobile and adaptable. In-Store Farms are located within retail spaces or restaurants, allowing for local and fresh food production near the point of consumption. Finally, Appliance Farms refer to compact vertical farming devices integrated into homes or offices, enabling convenient, personalized food production in limited spaces. Each type of vertical farm provides unique benefits tailored to specific food production and consumption needs.

In their book “*Plant Factory: An Indoor Vertical Farming System for Efficient Quality Food Production*,” (Kozai et al., 2019) define vertical farming as a multilayer indoor plant production system in which all growth factors—such as light, temperature, humidity, carbon dioxide (CO₂) concentration, water, and nutrients—are precisely controlled to produce large volumes of high-quality fresh produce year-round, fully independent from sunlight or outdoor conditions.

2.1.2 Concept of Vertical Farming

Vertical farming refers to the cultivation of plants in stacked layers or vertical structures within controlled environments that maximize spatial and resource efficiency (Benke & Tomkins, 2017). This technique is particularly relevant in urban contexts, where arable land is limited (Figure 1), and contributes to reducing the carbon footprint associated with food transportation (Beacham et al., 2019).

Additionally, vertical farming enables the regulation of key environmental parameters such as light, temperature, and humidity, thereby optimizing year-round production regardless of external climatic conditions (Kalantari et al., 2017).



Figure 1. A multi-level vertical farming system in an urban indoor setting.

2.1.3 NFT Hydroponic Systems: Principles and Operation

The Nutrient Film Technique (NFT) is a hydroponic method involving a continuous flow of a thin film of nutrient solution over the bare roots of plants (figure 2), enabling efficient absorption of nutrients and oxygen (Reddy et al., 2023).

Key principles of NFT:

- i. Direct exposure of roots to the nutrient solution.
- ii. Continuous and homogeneous nutrient flow to avoid accumulation or deficiency.

- iii. Recirculation of the solution to minimize water and fertilizer waste.

Typical operation:

- i. Plants are placed in holders over slightly inclined growing channels.
- ii. The nutrient solution is pumped from a reservoir to the upper end of the channels.
- iii. Gravity allows the solution to flow evenly, bathing the roots.
- iv. Unabsorbed solution returns to the tank for reuse.

Proper maintenance of parameters such as channel slope (1–3%) and controlled flow rate is critical to avoid water stress on the roots (Shrouf, 2017).

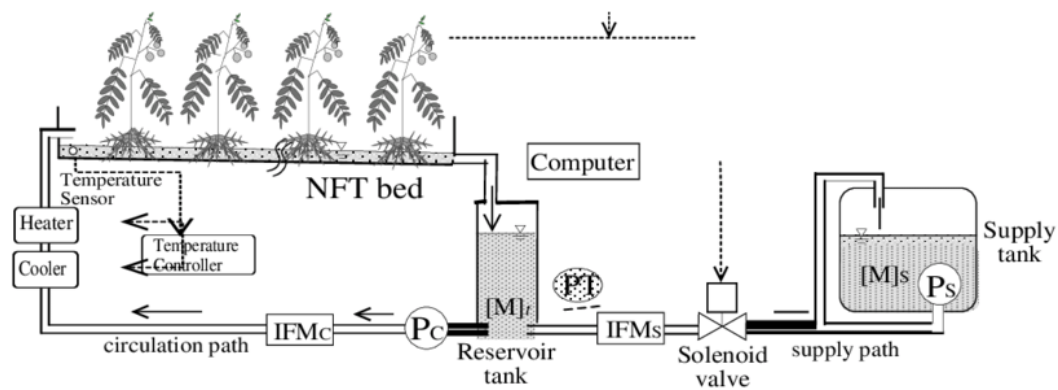


Figure 2. NFT system components and nutrient solution flow path (Falah, 2010).

2.1.4 Advantages and Limitations of NFT Systems

Advantages:

- i. Water and nutrient efficiency: NFT uses up to 90% less water than traditional methods (Al-Kodmany, 2018).
- ii. Accelerated growth: Constant access to oxygen and nutrients enhances plant development.
- iii. Reduced disease risk: The absence of soil lowers the incidence of root pathogens.

Limitations:

- i. Technical vulnerability: Any interruption (e.g., power outage) may threaten crops within hours (Kalantari et al., 2017).
- ii. Root clogging: Roots may obstruct channels without regular maintenance.

- iii. Crop restrictions: Not all species adapt well; plants with extensive root systems require modifications (Shrouf, 2017).
- iv. NFT systems offer a highly efficient and productive strategy within vertical farming, yet they demand constant monitoring and backup systems to prevent significant losses.

2.2 AUTOMATION AND BIOMECHATRONICS IN VERTICAL FARMING

2.2.1 Concepts of Applied Biomechatronics

Figure 3 shows applied biomechatronics in vertical farming refers to the integration of mechanical, electronic, computing, and control systems to optimize plant growth in regulated environments (Shamshiri et al., 2024). This field merges robotics, advanced sensing, and data analysis to automate key agricultural processes such as irrigation, fertilization, and climate control.

- i. Within the context of vertical farming, the goals of biomechatronics include:
- ii. Reducing human intervention while enhancing operational efficiency.
- iii. Ensuring optimal growth conditions through real-time control systems.
- iv. Dynamically adapting environmental factors to the physiological needs of different crops.

This discipline promotes a precision agriculture approach, where decisions are based on quantifiable data to maximize yield and minimize resource consumption.

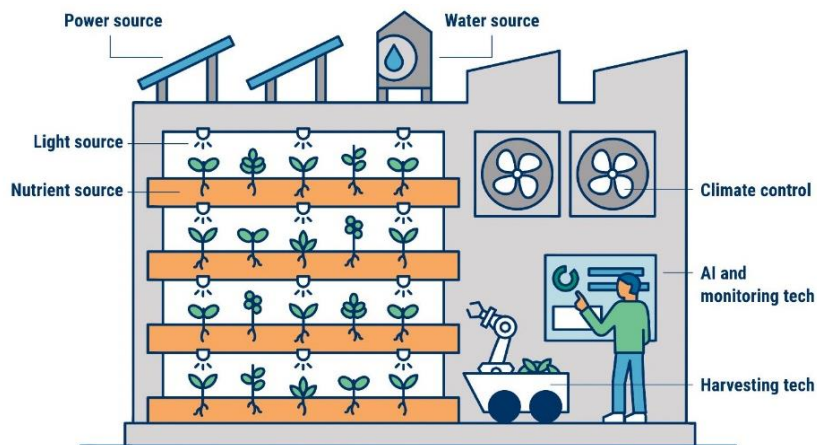


Figure 3.A biomechatronic-controlled vertical farming system (Tubbs, 2021).

2.2.2 Sensors and Controllers in Hydroponic Cultivation

Sensors and controllers are essential components in the automation of hydroponic systems, as they enable real-time monitoring and regulation of critical variables. According to (Narayan et al., 2024), key sensors include:

- i. **pH sensors:** Monitor the acidity or alkalinity of the nutrient solution, vital for nutrient uptake.
- ii. **Electrical Conductivity (EC) sensors:** Measure the concentration of dissolved salts and nutrients.
- iii. **Ambient temperature and humidity sensors:** Regulate the microclimate around plants.
- iv. **Water level sensors:** Ensure proper reservoir levels.

Controllers—typically microcontrollers such as Arduino or more advanced platforms like Raspberry Pi—process sensor data and execute automatic actions such as activating pumps, adjusting ventilation, or modifying LED lighting (Patel et al., 2016). Their synergy maintains system stability and enables dynamic optimization of the growing environment.

2.2.3 Monitoring Platforms: Grafana, IoT, and Big Data

The integration of digital platforms has transformed crop management in vertical farming:

- i. **Grafana** is a data visualization platform that enables real-time monitoring of variables like temperature, humidity, and pH through interactive web-accessible dashboards (Al-Garadi et al., 2018).
- ii. **Internet of Things (IoT)** connects sensors and actuators to local or cloud-based networks, allowing remote control of farming systems. It also enables automated alerts and immediate responses to environmental fluctuations (Dhanaraju et al., 2022).
- iii. **Big Data** refers to the large-scale analysis of sensor-generated data. With machine learning algorithms, it supports predictive insights for irrigation, fertilization, and climate adjustments, improving farming efficiency (Soussi et al., 2024).

The combination of these technologies drives sustainability, precision, and scalability in vertical farming, supporting the development of smart farms.

2.3 PLANT GROWTH PHYSIOLOGY IN HYDROPONIC CULTIVATION

2.3.1 Factors Affecting Growth of *Lactuca sativa*

In hydroponic systems, the growth of *Lactuca sativa* (lettuce) depends on physical, chemical, and biological factors interacting within a controlled environment (figure 4).:

- i. **Temperature:** Optimal range is 18–22 °C; temperatures above 26 °C can induce premature bolting and bitterness (Rouphael et al., 2012).
- ii. **Light intensity and quality:** LED lighting in the red-blue spectrum enhances photosynthesis and growth in lettuce.

- iii. **Nutrient concentration (EC):** EC levels between 1.5 and 2.5 mS/cm are ideal for maximizing yield (Yaakup et al., 2024).
- iv. **Solution pH:** Ideal pH is 5.5 to 6.5 to ensure efficient uptake of macro- and micronutrients.
- v. **Relative humidity:** Optimal range is 50% to 70%, which reduces fungal diseases and supports gas exchange.
- vi. **Root zone oxygenation:** Adequate oxygen supply prevents hypoxia and promotes robust root development (Goto et al., 1997).

The dynamic interaction of these factors determines photosynthetic rate, nutrient uptake, and ultimately, crop yield

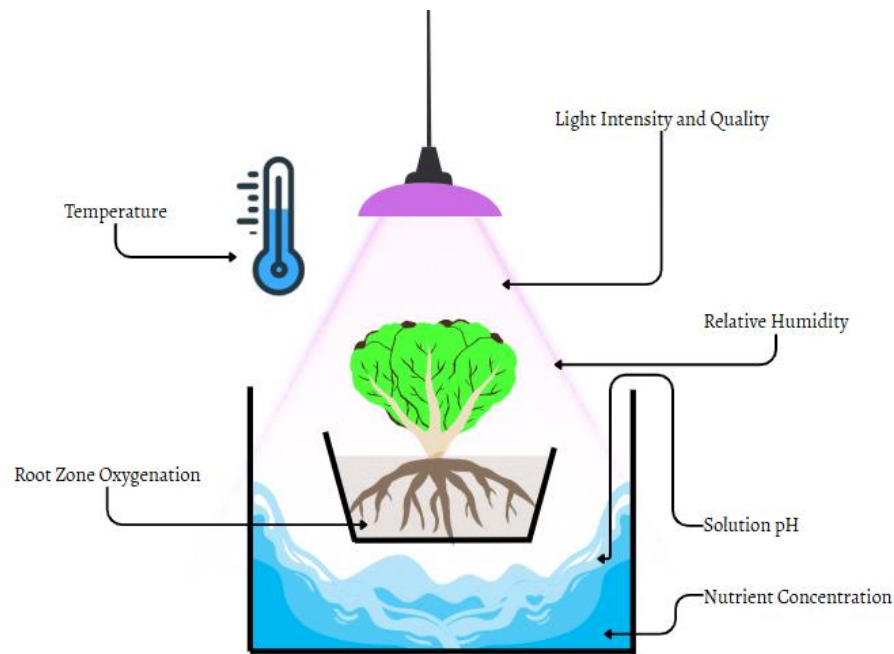


Figure 4.Environmental factors affecting hydroponic lettuce growth.

2.3.2 Role of Symbiotic Association with *Medicago sativa*

The symbiotic association between *Lactuca sativa* and *Medicago sativa* (alfalfa) represents an emerging strategy in sustainable and hydroponic cultivation. Traditionally, *Medicago sativa* is known for its nitrogen-fixing relationship with *Rhizobium* spp., but recent studies explore how its root exudates may benefit neighboring crops.

Potential benefits of co-cultivation:

- i. **Improved nitrogen availability:** Root exudates from *Medicago sativa* enrich the hydroponic medium with organic nitrogen compounds that *Lactuca sativa* can absorb (Chamkhi et al., 2022).
- ii. **Phytohormone stimulation:** Symbiosis may trigger the production of growth-promoting hormones such as auxins and cytokinins (Abusuwar & Omer, 2011; Jamil et al., 2022).
- iii. **Positive allelopathic effects:** Bioactive compounds from *Medicago sativa* can suppress root pathogens, fostering a healthier rhizosphere (Kong et al., 2024).

However, careful management of biomass ratios and co-cultivation conditions is necessary to avoid resource competition or nutrient imbalances. When properly regulated, this symbiotic association offers a promising path toward more efficient hydroponic production systems.

2.4 NITRATE AS A NUTRIENT AND ITS MANAGEMENT IN VERTICAL FARMING

2.4.1 Function of Nitrate in Plants

Nitrate (NO_3^-) is a major source of inorganic nitrogen for plants and plays a central role in their metabolism. Once absorbed, nitrate is reduced to ammonium (NH_4^+) and subsequently assimilated into amino acids, the building blocks of plant proteins (Zayed et al., 2023)

- i. **Key functions of nitrate:**
- ii. **Amino acid and protein synthesis:** Essential for cell growth and division.
- iii. **Chlorophyll production:** Nitrogen is a core component of chlorophyll, critical for photosynthesis.
- iv. **Growth regulation:** Nitrate acts as a molecular signal influencing root development, cell elongation, and leaf formation (Gao et al., 2022).

In hydroponics and vertical farming, proper nitrate availability and management are key to achieving optimal vegetative growth and high crop quality.

2.4.2 Effects of Nitrate Excess and Deficiency in Crops

Imbalances in nitrate supply can significantly impact crop development:

Nitrate deficiency:

- i. Chlorosis in older leaves due to nitrogen remobilization.
- ii. Stunted growth and reduced yield.
- iii. Delayed leaf and floral development (Miller et al., 2007).

Nitrate excess:

- i. Accumulation in plant tissues, affecting food quality and increasing the risk of toxic nitrosamine formation.
- ii. Excessive vegetative growth at the expense of reproductive structures.
- iii. Environmental consequences such as leaching and water source contamination (Bian et al., 2020).
- iv. Rational nitrate management enhances productivity and contributes to the sustainability of vertical farming systems.

2.4.3 Rhizosphere-Plant Interactions and Nitrogen Optimization

The rhizosphere, the zone surrounding the roots in soil or hydroponic media is a dynamic interface where complex interactions among plants, microbes, and nutrients occur. These interactions are critical for improving nitrogen use efficiency (NUE) (Olanrewaju & Babalola, 2022).

Strategies to optimize nitrogen use in vertical farming:

- i. **Biofertilizers and Plant Growth-Promoting Rhizobacteria (PGPR):** Microorganisms like *Azospirillum* and *Rhizobium* enhance nitrogen availability and root uptake (Faran et al., 2023).
- ii. **Nitrate concentration adjustment:** Real-time EC monitoring prevents over- or under-supply.
- iii. **Precision agriculture technologies:** IoT and Big Data platforms enable accurate predictions of nitrogen needs.

Modern approaches favor adaptive, physiology-based fertilization strategies guided by real-time environmental and rhizospheric data.

2.5 PHOTOSYNTHESIS AND ENERGY CONVERSION EFFICIENCY IN CROPS

2.5.1 Overview of Photosynthesis

Photosynthesis is the biochemical process through which plants convert light energy into chemical energy, producing carbohydrates essential for their growth and releasing oxygen as a byproduct. It takes place in chloroplasts through two primary stages: the light-dependent reactions and the Calvin cycle (figure 5), (Xu et al., 2021).

Photosynthetic efficiency depends on various factors:

- i. Light intensity and quality

- ii. Carbon dioxide (CO₂) availability
- iii. Temperature
- iv. Plant water and nutrient status

In controlled environments such as vertical farming, these factors are meticulously regulated to maximize photosynthetic efficiency and crop yield (Wu et al., 2025).

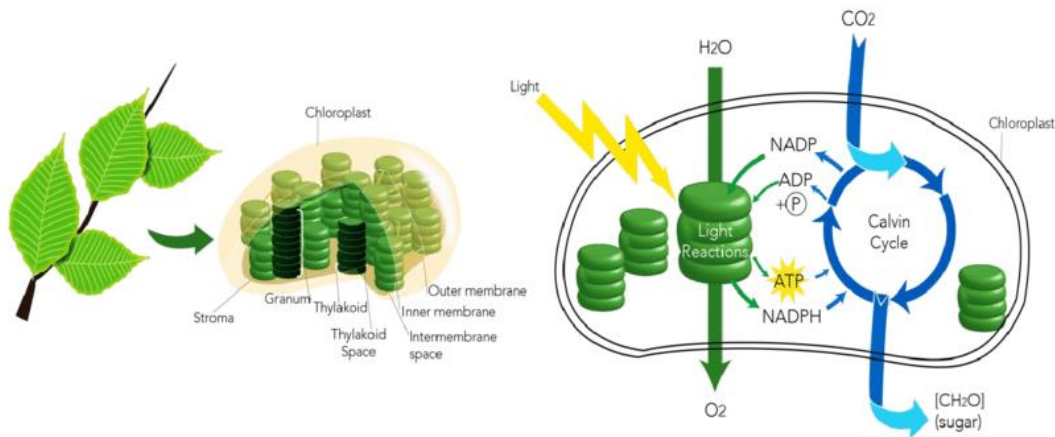


Figure 5. Photosynthesis process (Karakaya et al., 2021)

2.5.2 Dynamics of Artificial Light in Vertical Farming

Artificial lighting primarily LED is widely used in vertical farming systems to supplement or replace natural sunlight. LEDs allow precise control of both light intensity and spectrum (Figure 6), which are crucial for optimizing photosynthesis (SharathKumar et al., 2020).

Key elements of artificial light dynamics:

- i. **Light spectrum:** Red light (~660 nm) promotes photosynthesis and flowering, while blue light (~450 nm) supports vegetative development and the production of secondary metabolites (Bantis et al., 2018; Mickens et al., 2018).
- ii. **Photoperiod:** The duration of light-dark cycles directly influences plant morphogenesis and metabolism.
- iii. **Light intensity (PPFD, $\mu\text{mol}/\text{m}^2/\text{s}$):** Maintaining proper photon flux density is essential to prevent light saturation or stress.

Modulating these parameters has led to improvements in energy conversion efficiency and reductions in energy costs per biomass unit.

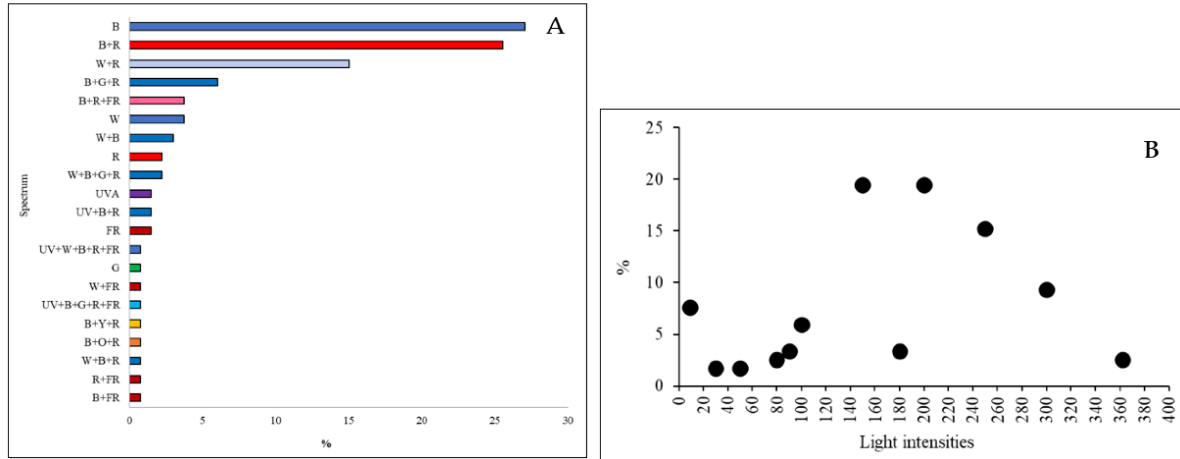


Figure 6. LED lighting systems with spectrum distribution used in vertical farms. (A) and light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (B) used in the analysed research. Data are expressed as percentage ($n = 118$). B (blue 450–495 nm); R (red 620–700 nm); FR (far-red 700–800 nm); G (green 500–560 nm); O (orange 600–620). Adapted from: (Nájera et al., 2022)

2.5.3 Relationship Between Lighting and Agronomic Performance

The relationship between light quality, quantity, duration and agronomic performance is direct and significant:

- A balanced spectrum not only increases total biomass but also enhances nutritional quality (Li et al., 2022).
- Optimizing PPFD can boost net photosynthesis by up to 30%, particularly in species like *Lactuca sativa* (Kozai et al., 2015).
- Light Use Efficiency (LUE) is a key indicator of how much biomass is produced per unit of absorbed light energy. Improving LUE leads to higher yields with lower energy inputs.
- Smart lighting management is thus essential for both economic and environmental sustainability in vertical farming.

2.6 PHOTOSYSTEM II (PSII) AND SOLAR ENERGY CAPTURE

2.6.1 Molecular Structure of PSII

Photosystem II (PSII) is a large multi-protein complex embedded in the thylakoid membranes of chloroplasts, essential for the light-dependent phase of photosynthesis (figure 7). Its primary function is to capture solar energy and initiate the electron transport chain (Umena et al., 2011).

Key structural components of PSII include:

- Protein complexes:** Mainly D1 and D2, forming the reaction center
- Pigments:** Chlorophyll a, chlorophyll b, and carotenoids to absorb light
- Cofactors:** Pheophytin, plastoquinone, and a manganese cluster (Mn_4CaO_5) for water splitting
- Oxygen Evolving Complex (OEC):** Catalyzes the oxidation of water into O_2 , protons, and electrons (Ifuku & Noguchi, 2016).

PSII's highly organized structure allows efficient energy transfer from captured photons to chemical reaction centers.

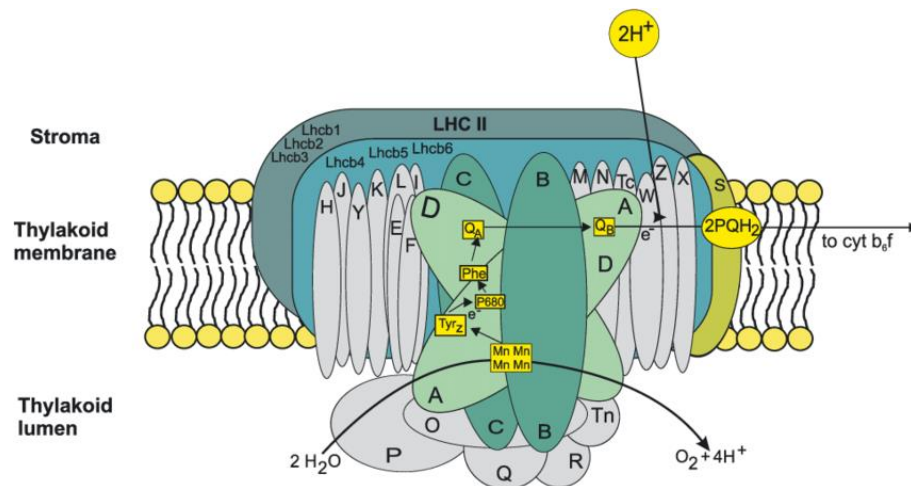


Figure 7. Schematic model of structural organization of PSII within thylakoid membrane (Luciński & Jackowski, 2006)

2.6.2 Photon Capture and Electron Transport Mechanisms

The process of photon capture and electron transport in PSII involves several key steps:

- Light absorption:** Antenna pigments absorb photons and transfer the excitation energy to P680.

- ii. **P680 excitation:** P680, a special chlorophyll a molecule, becomes excited and donates an electron to pheophytin.
- iii. **Electron transport:** Excited electrons pass sequentially to plastoquinone QA and QB.
- iv. **Water photolysis:** PSII splits water molecules to replenish lost electrons, releasing oxygen and protons (Muhammad et al., 2021; Shuang et al., 2022).

These electrons continue to Photosystem I, establishing a proton gradient that drives ATP synthesis.

2.6.3 PSII Vulnerability to Stress Factors

Despite its efficiency, PSII is highly sensitive to environmental stresses that may cause damage or inactivation:

- i. **Light stress:** Excess light leads to photoinhibition, damaging the D1 reaction center and lowering efficiency (Violet-Chabrand et al., 2017).
- ii. **Extreme temperatures:** Affect thylakoid membrane fluidity and electron transport stability.
- iii. **Water deficit:** Limits photolysis, reducing electron availability.
- iv. **Pollutants:** Heavy metals and oxidative agents can inactivate PSII components (Dong et al., 2019).

Improving PSII resilience is a central goal in current plant biotechnology, especially for controlled agriculture applications like vertical farming.

2.7 CHLOROPHYLL FLUORESCENCE AS A PHYSIOLOGICAL INDICATOR

2.7.1 Physical Principles of Chlorophyll Fluorescence

Chlorophyll fluorescence refers to the re-emission of absorbed light at longer wavelengths (around 680 nm) when not fully utilized in photochemistry (figure 8). It occurs when absorbed energy is not used in water photolysis or electron transport (Murchie & Lawson, 2013).

Physically:

- i. Unused light energy is dissipated as heat or re-emitted as fluorescence.
- ii. Fluorescence analysis provides indirect insights into PSII's functional state and early signs of physiological stress.

This non-invasive technique has been widely adopted in precision agriculture and vertical farming to monitor plant health.

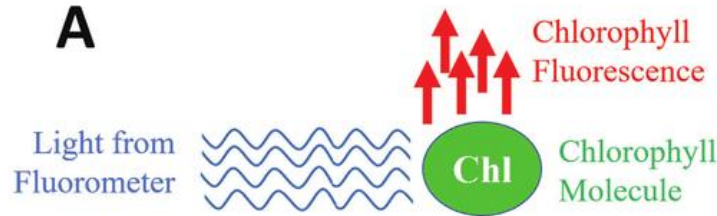


Figure 8. Adapted diagram showing the principles of chlorophyll fluorescence (Part A only) (Cangelosi et al., 2019).

2.7.2 Key Parameters: Fv/Fm, Φ PSII, ETR, NPQ

Critical parameters from chlorophyll fluorescence analysis include:

- i. **Fv/Fm (maximum quantum efficiency):** Reflects the PSII potential under non-stress conditions. Optimal values near 0.83; lower values indicate stress or photoinhibition (Kalaji et al., 2018).
- ii. **Φ PSII (effective quantum yield):** Measures real-time electron transport efficiency under light.
- iii. **ETR (Electron Transport Rate):** Indicates the speed of electron movement through PSII.
- iv. **NPQ (Non-Photochemical Quenching):** Represents energy dissipated as heat for photoprotection (Ruban, 2016).

Together, these parameters reveal both baseline and dynamic photosynthetic responses.

2.7.3 Measurement Techniques: PAM Fluorometry

PAM Fluorometry (Pulse-Amplitude-Modulated) is the gold standard for quantitative fluorescence measurement. It involves:

- i. A weak measuring pulse to detect base fluorescence (F_0)
- ii. A saturation pulse to determine maximum fluorescence (F_m)
- iii. Actinic light to stimulate photosynthesis during dynamic readings

Advantages of PAM:

- i. Enables measurement under real (light-active) conditions

- ii. Provides real-time values for F_v/F_m , Φ_{PSII} , ETR, NPQ
- iii. Highly sensitive to early changes before visible symptoms (Guidi & Calatayud, 2013).

Its use has expanded in research and real-time crop monitoring in vertical farms and advanced hydroponics.

2.8 NITRATE STRESS AND PHOTOSYNTHETIC EFFICIENCY

2.8.1 Effects on Φ_{PSII} under Variable Nitrogen Conditions

Nitrate stress—whether deficiency or excess—directly affects the effective quantum yield of PSII (Φ_{PSII}), a key metric of light-to-chemical energy conversion efficiency.

- i. **Nitrate deficiency:** Reduces chlorophyll and protein synthesis, limiting light capture and electron transport, resulting in decreased Φ_{PSII} and photosynthetic output (Mu & Chen, 2021).
- ii. **Nitrate excess:** Initially stimulates vegetative growth but causes ionic imbalance and osmotic stress, reducing Φ_{PSII} and inducing photooxidative damage (Ru et al., 2024).

Φ_{PSII} fluctuations serve as early stress indicators—critical for hydroponics and vertical farming systems.

2.8.2 Case Studies in *Lactuca sativa* and *Medicago sativa*

Recent studies show how nitrogen availability impacts photosynthetic efficiency in crops like *Lactuca sativa* and *Medicago sativa*: In *Lactuca sativa*, suboptimal nitrate reduces Φ_{PSII} and ETR, decreasing biomass and nutritional quality (Yaakup et al., 2024). Excess nitrate increases tissue accumulation, lowers NPQ, and heightens light stress sensitivity. In *Medicago sativa*, its symbiosis with nitrogen-fixing rhizobacteria grants resilience to low external nitrate. However, high nitrate availability may suppress this symbiosis, reducing Φ_{PSII} and F_v/F_m (Sun et al., 2025). These cases highlight the importance of precise nitrogen management in vertical farming to optimize photosynthesis.

2.9 INTEGRATING Φ_{PSII} MEASUREMENTS IN BIOMECHATRONIC AGRICULTURE

2.9.1 In-Situ PAM Technologies

PAM technologies have evolved into portable, in-situ devices, enabling real-time photosynthetic assessments directly in the crop system.

Key features:

- i. **Non-destructive monitoring:** Evaluates plant function without damage

- ii. **Dynamic measurement:** Tracks fast variations in Φ PSII, Fv/Fm, NPQ under diverse conditions (Keates et al., 2019; Pandey & Paul, 2014)
- iii. **IoT compatibility:** New PAM devices are integrated with data platforms for remote storage, analysis, and visualization

These advances provide objective physiological indicators for automated climate and nutrient control.

2.9.2 Automation of Nutritional Stress Monitoring

Integrating PAM sensors into agricultural IoT networks allows for automated monitoring of nutrient-related stress, especially nitrogen stress.

Main benefits:

- i. **Early deficiency detection:** Changes in Φ PSII signal imbalances before visual symptoms (Kalaji et al., 2018).
- ii. **Automated adjustments:** Smart systems can correct nutrient supply (e.g., nitrate levels) based on Φ PSII thresholds
- iii. **Input reduction:** Optimizing fertilizer use enhances sustainability

This integration enhances precision agriculture and productivity in advanced hydroponic systems.

2.9.3 Dynamic Control Applications in NFT Systems

In NFT (Nutrient Film Technique) systems, dynamic control based on Φ PSII measurements offers major benefits:

- i. **Nutrient flow optimization:** Adjusting solution speed and composition according to real-time crop demand (Kim et al., 2024)
- ii. **Lighting and irrigation regulation:** LED intensity and irrigation frequency can be automated based on photosynthetic efficiency
- iii. **Minimized water and nutrient stress:** Dynamic responses maintain balance for optimal growth and quality

This combination of physiological monitoring and advanced biomechatronics enables the development of intelligent vertical farms, where each plant receives precisely what it needs in real time.

2.10 STATE OF THE ART.

The need for sustainable, high-efficiency food production systems has intensified due to global population growth, urban expansion, and the adverse impacts of climate change. In response, Controlled-Environment

Agriculture (CEA) has emerged as a promising solution, offering tightly regulated conditions for crop growth by isolating them from external climatic variability and enabling optimal use of water, energy, and nutrients (Chaves, 2002; Verma et al., 2024; Zhu & Marcelis, 2023). Within this context, Vertical Farming (VF) represents a cutting-edge innovation that maximizes spatial efficiency by utilizing stacked growing systems combined with advanced technologies such as LED lighting, hydroponics, and automated environmental regulation (Kumar et al., 2025; Malik, 2024). This configuration allows for continuous year-round production of crops such as *Lactuca sativa* (lettuce), particularly in urban settings, where space and resources are limited.

Among the hydroponic techniques used in VF systems, the Nutrient Film Technique (NFT) stands out for its capacity to provide a continuous flow of nutrient solution, which enhances oxygen availability at the root zone and allows for precise control of nutrient composition and pH (Rajalakshmi & Gunasekaran, 2024; Resh, 2016). Despite its benefits, there is still a limited body of research addressing how nitrate (NO_3^-) concentration gradients affect physiological responses in NFT systems, especially when intercropped with legumes like *Medicago sativa* (alfalfa).

Nitrogen is a central macronutrient involved in the biosynthesis of chlorophyll, amino acids, and nucleic acids, making it essential for plant development. However, improper nitrate management in hydroponic systems—especially under suboptimal light conditions—can result in excessive NO_3^- accumulation in edible tissues and altered plant metabolism (Brazaitytė et al., 2021; Demšar et al., 2004; Fathi, 2022; Pomoni et al., 2023). Vertical farms, by virtue of their technological sophistication, offer an ideal environment to test different nitrate regimes and their effects on photosynthetic performance, biomass production, and nutrient uptake in crops like lettuce. Yet, most studies have focused on static or batch hydroponic systems, with little emphasis on dynamic or continuous-flow NFT systems (Breš et al., 2022; Holatko et al., 2025; Martínez-Moreno et al., 2024; Soufi et al., 2023). This creates a pressing need to evaluate fixed nitrate concentrations in NFT under controlled conditions.

Additionally, **intercropping strategies**, particularly the use of legumes such as *Medicago sativa*, present a viable agroecological approach to improve nitrogen use efficiency and reduce dependency on synthetic fertilizers. Legumes are capable of **biological nitrogen fixation (BNF)** via symbiosis with *Rhizobium* species, contributing to improved soil fertility and crop productivity (Ju et al., 2019; Matse et al., 2020; Swarnalakshmi et al., 2020). Field and greenhouse experiments have demonstrated that co-cultivating lettuce with alfalfa improves biomass production, nutrient uptake (especially potassium and calcium), and post-harvest quality, while reducing nitrate accumulation (Pérez Gómez & Pineda Pineda Joel, 2023; C. Wang et al., 2022; L. Zhang et al., 2024). However, these findings remain largely restricted to soil-based or passive hydroponic systems, and their extrapolation to high-precision, automated NFT systems is still limited.

In more advanced studies, researchers have begun to document the physiological interactions between *Lactuca sativa* and *Medicago sativa* under stress conditions, such as salinity or heavy metal contamination.

Notably, intercropping was shown to enhance lettuce antioxidant responses and modify the rhizosphere microenvironment in ways that increase tolerance to external stressors like lead (Pb), cadmium (Cd), and nanoparticles (Guerchi et al., 2024; Morsy et al., 2022; L. Zhao et al., 2016). These observations suggest that intercropping may improve plant resilience beyond nutrient management.

A critical advancement in modern VF systems is the integration of biomechatronic technologies. These systems combine sensors, actuators, and control units (such as ESP32, Raspberry Pi, or Arduino) to automatically regulate environmental parameters—temperature, humidity, CO₂, light, and irrigation—in real time (Laddha, 2023; Mendon et al., 2022; Vineeth & Ananthan, 2023). Several studies report increased water-use efficiency and biomass gains of up to 30% through the automated control of environmental variables like pH, EC, and photoperiod (Kaur & Jain, 2024; L. Pagaduan et al., 2024). Despite this progress, many systems still lack feedback based on physiological plant status, relying instead on static setpoints rather than dynamic adjustment informed by photosynthetic performance (Duangpakdee & Sukpancharoen, 2024).

An emerging area of interest is the real-time monitoring of Photosystem II (PSII) using non-destructive tools such as the JUNIOR-PAM fluorometer. Key indicators like the maximum quantum yield (Fv/Fm), effective quantum yield (Φ PSII or Y(II)), and non-photochemical quenching (NPQ) are now widely accepted as sensitive markers of light stress and photosynthetic efficiency (Ajigboye et al., 2014; Maxwell & Johnson, 2000; Tian et al., 2017; W. J. Wang et al., 2018). Incorporating these variables into cyber-physical farming systems enhances the ability to adapt lighting, CO₂ supply, and nutrient management to the metabolic state of the plant in real time (Lanoue et al., 2024).

In their study, (Vaštakaitė-Kairienė & Viršilė, 2015) aimed to determine changes in the mineral nutrient content of Brassicaceae microgreens depending on different blue-to-red (B:R) light ratios using LED lighting, as well as to assess their growth and nutritional value through multiple indices. Their results showed that a higher percentage of blue light (B75R25 and B100R0) positively influenced the accumulation of mineral nutrients in Brassicaceae microgreens, suggesting that such light conditions could be strategically used as a tool for biofortification. However, blue-dominant light also resulted in lower yield and shorter hypocotyls.

In their article, (Ptushenko et al., 2020) noted that the diversity of plant responses to light spectrum variation arises from three major factors: (i) activation of specific photoreceptors, (ii) uneven efficiency of different spectral components in driving photosynthesis, and (iii) variable light penetration depth into the leaf. Their study illustrated how these factors influence chloroplast photorelocation movements and spectral light capture, which are closely linked to photosynthetic capacity.

In 2020, (Hyun Kang et al., 2020) from the Department of Plant Sciences in Korea explored how plants perceive and respond to light. They tested whether plant light perception could be quantified based on the

absorption by their main photoreceptors: phytochrome, cryptochrome, and phototropin. Using different lamps with blue and red spectra, they concluded that artificial lighting could be made more efficient by modifying and adjusting spectral quality. Their findings also confirmed that the red-absorbing form of phytochrome is a valid experimental variable for assessing light quality in plant studies. These results highlight the possibility of modulating plant growth responses through tailored artificial light spectra.

In a study by (Jayalath & van Iersel, 2021; Liu et al., 2021), light absorption was measured under various monochromatic and mixed-color treatments (80R20A, 80V20A, 80A20V, 80R20V, and 16A20V64R). Results showed that absorption was highest under red and blue light (>0.9 UR), and lowest under green light (0.811 UR), with mixed spectra showing intermediate absorption values.

Consequently, indirect chlorophyll quantification has become a reliable tool with significant potential for guiding nitrogen fertilization strategies in crop management (de Lima Vasconcelos et al., 2014). Chlorophyll readings are typically performed weekly, one hour before the end of the DLI, focusing on the middle third of the leaf blade (Breeuwer et al., 2008). According (de Lima Vasconcelos et al., 2014), chlorophyll content measurements have a direct correlation of 99% with chlorophyll (mg/g) and 97% with nitrogen content (%) under two mathematical models he proposed.

Furthermore, energy-related indicators such as Light Use Efficiency (LUE) and Energy Use Efficiency (EUE) provide quantitative metrics for evaluating the sustainability of vertical farming systems. Standard LUE values for lettuce range between 0.56 and 0.75 g DW mol⁻¹ of photosynthetically active radiation, while EUE values are typically below 1.2 g FW kWh⁻¹. Optimizing PPFD within the range of 250–300 μmol m⁻² s⁻¹ has been shown to improve these efficiencies (Hostalrich et al., 2022; Hytönen et al., 2018; Jayalath & van Iersel, 2021; Nelson & Bugbee, 2014). However, these indicators are often studied in isolation, without correlating them to physiological or interspecific interactions.

In summary, while vertical farming and NFT systems offer exceptional control and productivity potential, key research gaps persist regarding: The interaction between fixed nitrate concentrations and photosynthetic responses in continuous-flow NFT; "The influence of *Medicago sativa* on *Lactuca sativa* performance under biomechatronically regulated environments; The integration of physiological feedback into automated farming systems for dynamic decision-making.

This thesis addresses these gaps by analyzing how *Lactuca sativa* responds to different nitrate concentrations under NFT conditions, when cultivated in association with *Medicago sativa*. It evaluates photosynthetic efficiency, nutrient uptake, energy use, and growth metrics under a fully automated, sensor-integrated vertical farming system.

III. METHODOLOGY

Figure 9 illustrates the experimental design used to evaluate the effects of different nitrogen concentrations on the growth of *Lactuca sativa* var. *longifolia* in a hydroponic Nutrient Film Technique (NFT) system, in coculture with *Medicago sativa* (alfalfa). This experiment was conducted under artificial lighting and automated environmental control. *Medicago sativa* was sown and treated with nitrogen-fixing bacteria at three specific stages: pre-planting (10^6 CFU/mL), the second week post-transplanting (10^8 CFU/mL), and the fourth week (10^6 CFU/mL). *Lactuca sativa* seedlings, commercially acquired at three weeks of age, were acclimatized in a nursery system for one week prior to co-transplantation. Both species were then transplanted into NFT channels, under controlled LED lighting conditions ($270 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$), and were exposed to nutrient solutions with varying nitrogen concentrations. Six treatments (T1–T6) were established, representing nitrogen levels of 0%, 33%, 66%, 100%, and 133%.

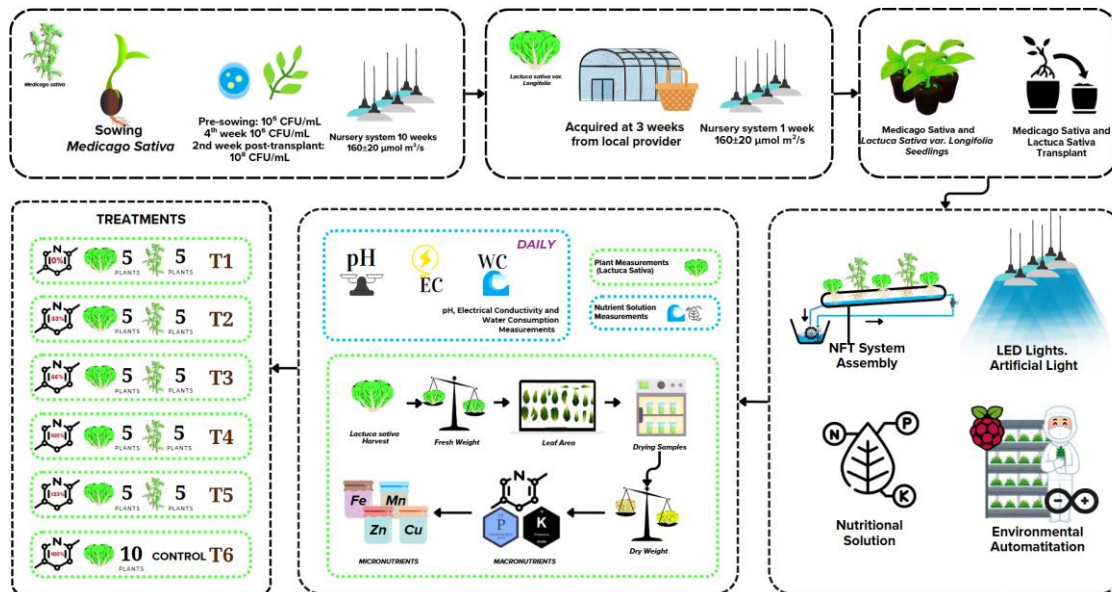


Figure 9. General scheme of the experiment for growing.

Treatments T1 to T5 were implemented as co-cultures of lettuce and alfalfa (5 plants of each species per treatment), while T6 served as the control, consisting of a monoculture of lettuce (10 plants) under 100% nitrogen. Throughout the experiment, pH, electrical conductivity (EC), and water consumption (WC) of the nutrient solution were monitored daily. At the end of the cycle, the response variables for *Lactuca sativa* included fresh weight, leaf area (measured via digital scanning), dry weight (measured after dehydration), and tissue concentrations of macro and micro-nutrients. This experimental design allowed for the assessment of the nutritional and physiological

efficiency of romaine lettuce when cultivated in association with alfalfa and subjected to varying nitrogen regimes. The study incorporated automated environmental controls and precise methods for quantifying growth.

3.1 PLANT MATERIAL

Three-week-old seedlings of lettuce (*Lactuca sativa* L. Longifolia, LS) were sourced from a local provider in Asientos, Aguascalientes, Mexico. These plants were kept in a nursery system for one week under light conditions of $160 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$ before being transplanted into the hydroponic system. Certified seeds of *Medicago sativa* (MS) variety San Miguelito, which had germination and purity rates of 90% and 98%, respectively, were sown in a 60-cavity black plastic seedbed measuring 20 x 60 x 5 cm. The MS plants were transplanted into the same hydroponic system at the tenth week, achieving a height of 25 ± 5 cm after their first cut.

Before sowing, the MS seeds were immersed in a suspension of *Rhizobium* at a concentration of 10^6 colony-forming units CFU mL⁻¹. The MS seedlings were also ex-posed to *Rhizobium* again in the second and fourth weeks after germination at the same concentration. Finally, in the second week after transplanting, *Rhizobium* was supplied at a concentration of 10^8 CFU mL⁻¹ to ensure adequate colonization and symbiosis with the plants

3.2 BIOFERTILIZERS USED IN THE EXPERIMENT

Five experimental conditions were established based on nutrient solution balances to modify nitrate levels (see Table 1). Municipal tap water from TecNM | Campus Pabellon de Arteaga was analyzed to determine its anionic and cationic composition. This analysis was conducted by Fertilab | Agricultural Laboratory, located in Cd. Industrial de Celaya, Celaya, Gto, Mx. Based on these results, a suitable nutrient solution balance for both plant species *Lactuca Sativa* (LS) and *Medicago sativa* (MS) was formulated. All solutions were balanced to maintain an electrical conductivity (EC) of 2.0 ± 0.5 mS cm⁻¹ and a pH of 6.0 ± 0.5 .

Table 1. Distribution of treatments and relative percentage of NO₃⁻ used

Treatments	NO ₃ ⁻ (%)	Plants
		(% Individuals)
T1	0	50- MS, 50-LS
T2	33	50-MS, 50-LS
T3	66	50-MS, 50-LS
T4	100	50- MS, 50-LS
T5	133	50-MS, 50-LS

T6 (Control)	100	100- LS
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3.3 PLANT NUTRITION

Table 2 summarizes the nutrient solution formulations corresponding to each nitrate proportion used in the experiment. All solutions were prepared in 50 L containers, and the nutrient composition was carefully adjusted to maintain cationic balance (Steiner, 1984), ensuring that variations in nitrate levels did not alter the electrical neutrality of the solution.

Table 2. Distribution of treatments and relative nitrate (NO_3^-) supply used in each experimental group.

Ions (mEq L^{-1})		Treatments.				
		T1	T2	T3	T4-T6	T5
MACRONUTRIENTS	KNO_3	0.00	0.00	0.00	3.00	6.50
	$\text{Ca}(\text{NO}_3)_2$	0.00	4.00	8.00	9.00	9.00
	HNO_3	0.00	0.90	0.90	0.90	0.90
	KH_2PO_4	7.00	6.50	4.50	1.00	0.50
	K_2SO_4	0.00	0.50	2.50	3.00	0.00
	CaSO_4	9.00	5.00	1.00	0.00	0.00
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	4.00	4.00	4.00	4.00	3.50
	H_2SO_4	0.90	0.00	0.00	0.00	0.00
MICRONUTRIENTS	FeSO_4	0.1430	0.1430	0.1430	0.1430	0.1430
	CuSO_4	0.0194	0.0194	0.0194	0.0194	0.0194
	MnSO_4	0.0031	0.0031	0.0031	0.0031	0.0031
	ZnSO_4	0.0100	0.0100	0.0100	0.0100	0.0100
	$\text{Na}_2 [\text{B}_4\text{O}_5 (\text{OH})_4]$	0.0065	0.0065	0.0065	0.0065	0.0065
	Na_2MoO_4	0.0030	0.0030	0.0030	0.0030	0.0030

3.4 BIOMECHATRONIC ENVIRONMENT AND REGULATION OF THE GROWING ENVIRONMENT

3.4.1 Overview.

The study was conducted in an automated vertical farm configured as a biomechatronic environment, designed to precisely regulate the microenvironmental conditions required for the physiological development of *Lactuca sativa* (LS) in association with *Medicago sativa* (MS). The system integrated distributed sensors, actuators,

and electronic modules capable of real-time monitoring and control of critical variables such as temperature, relative humidity, CO₂ concentration, nutrient solution flow, and light intensity. All functions were coordinated by a central control unit, which automatically managed the various subsystems, including lighting, ventilation, irrigation, and CO₂ enrichment. This architecture ensured the stability and reproducibility of experimental conditions, providing a controlled environment essential for accurately evaluating the physiological responses of both species to different nutritional treatments (figure 10).

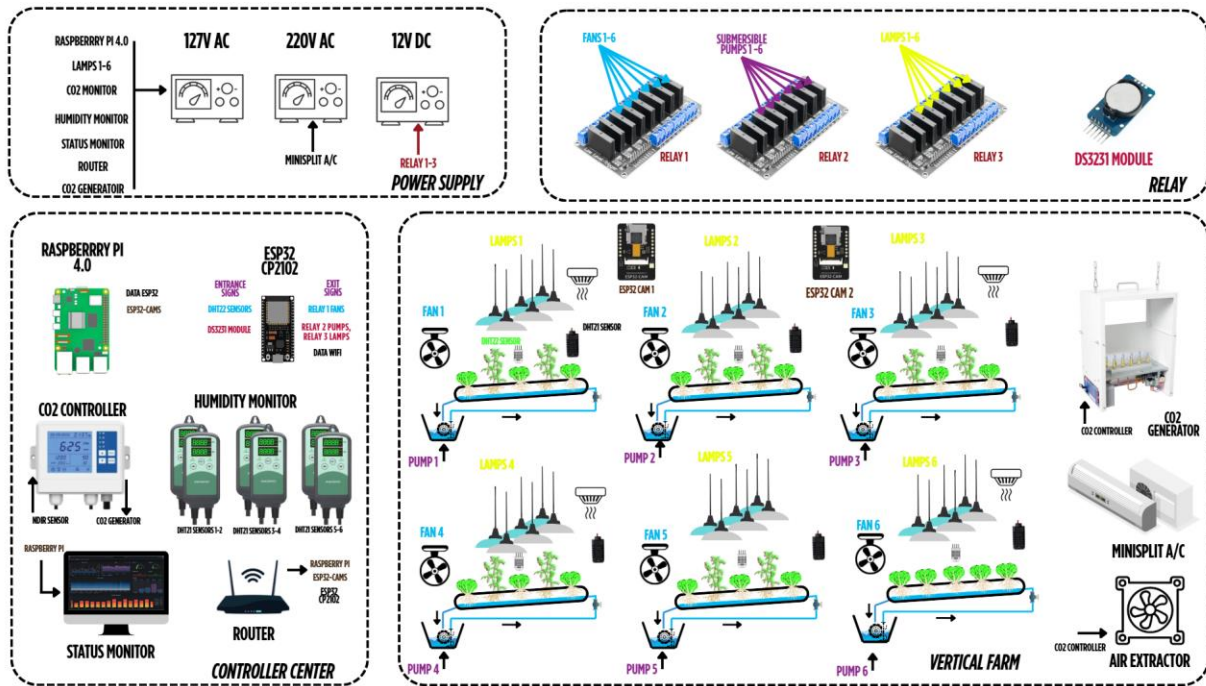


Figure 10. Diagram of the biomechatronic vertical farming system, showing the integration of sensors, actuators, and a control unit for real-time regulation of environmental variables.

Figure 11 illustrates the logical sequence of operation of the biomechatronic control system used in the vertical farming platform. The process begins with system activation and verification of Wi-Fi connectivity. Upon successful connection, a Raspberry Pi 4.0B unit communicates with multiple ESP32 modules responsible for acquiring data from environmental sensors and controlling corresponding actuators.

CO₂ levels are monitored through NDIR sensors, triggering a CO₂ generator when concentrations fall below 1000 ppm. Gas leaks and smoke are detected via MQ6 sensors, activating the air extractor if levels exceed 1300 ppm. Humidity and temperature are monitored by DHT21 sensors, which activate ventilation through relay-controlled fans if values exceed defined thresholds (e.g., >22°C or >70% RH).

Additionally, DHT22 sensors provide real-time data to regulate both temperature and humidity, while the DS3231 timing module manages light cycles and pump activation. Lighting control is executed through relay boards operating two lighting groups (white and blue LEDs), scheduled between 18:00 and 10:00. Climate stability is maintained by a minisplit system configured to 20°C and 65% relative humidity. The entire system is monitored remotely via a web server, with visual crop data collected by ESP32-CAM modules

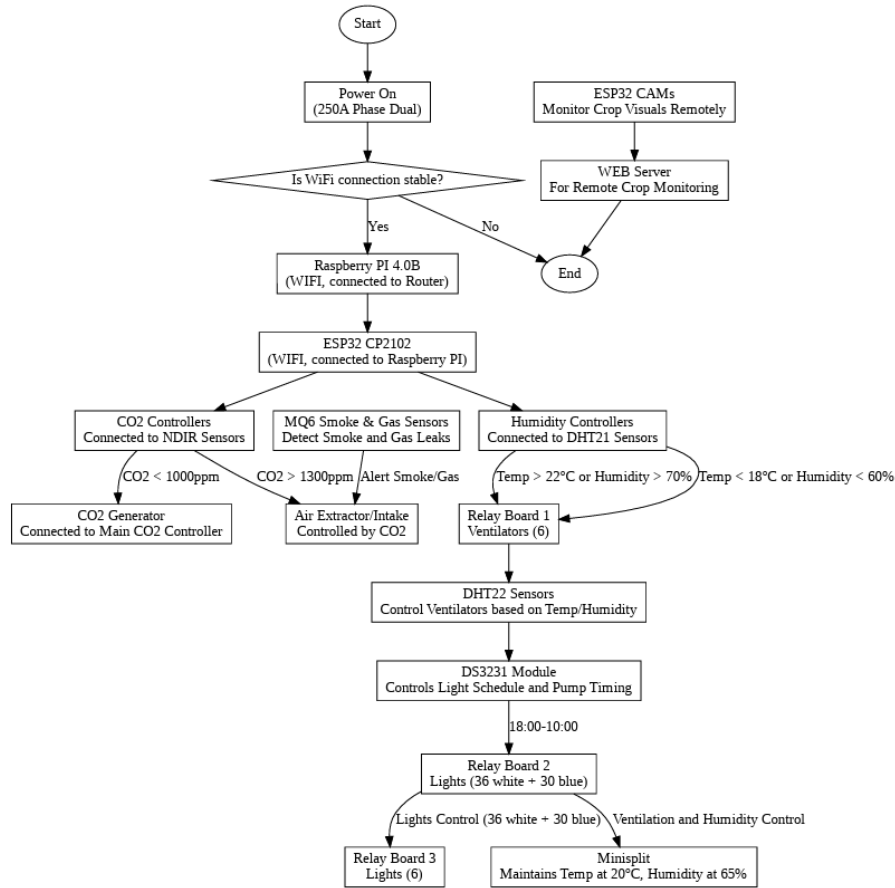


Figure 11. Sequence of operations for environmental monitoring and control in the vertical farming system, including CO₂, temperature, humidity, lighting, and ventilation regulation.

3.4.2 Temperature and Humidity

Thermal and humidity regulation was achieved using an automated system controlled by DHT21 and DHT22 sensors integrated into ESP32 microcontrollers connected to the central Raspberry Pi 4.0B unit. These sensors enabled real-time monitoring of the internal environmental conditions, triggering actuators based on predefined thresholds. When the temperature exceeded 22 °C or the relative humidity surpassed 70%, ventilation fans connected to Relay Board 1 were automatically activated to facilitate air exchange.

Under low-temperature conditions ($<18^{\circ}\text{C}$) or low humidity ($<60\%$), the system activated corrective mechanisms, including the operation of the minisplit, which maintained a stable temperature of 20°C and relative humidity of 65%. This configuration ensured constant environmental conditions suitable for the optimal growth and physiology of both plant species.

3.4.3 CO_2

Carbon dioxide (CO_2) concentration was monitored and regulated using three DANOPLUS AZ-7530 controllers (DANOPLUS, Taiwan), connected to high-precision NDIR sensors (Senseair, Sweden). These devices allowed continuous measurement of CO_2 levels within the cultivation environment, adjusting the concentration between 1000 and 1300 ppm to promote photosynthetic efficiency. When CO_2 concentrations fell below the target threshold, a VEVOR W4B-LP (VEVOR, China) CO_2 generator was automatically activated, increasing CO_2 levels to approximately 1250 ppm. In case of excessive accumulation (concentrations above 1300 ppm), the system activated air extraction and intake modules (intractor/extractor) to restore safe and physiologically optimal CO_2 levels. This feedback mechanism maintained stable atmospheric conditions throughout the experimental period.

3.4.2 Artificial Light System Characteristics

The artificial light environment was created using a combination of two LED light sources. One white LED produced a photosynthetic photon flux density (PPFD) of $200 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$, while a blue LED provided a PPFD of $60 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$. The PPFD was measured with a LightScout Solar Electric Quantum Meter from Spectrum Technologies, Inc. The emission spectra of the LEDs are illustrated in Figure 12. These light spectra were recorded using a RedTide spectrophotometer from Ocean Optics. The photoperiod was set to 16 hours per day, and the total PPFD achieved was $260 \pm 40 \mu\text{mol m}^{-2} \text{s}^{-1}$, resulting in a Daily Light Integral (DLI) of $14.976 \pm 2.3 \text{ mol photons m}^{-2} \text{d}^{-1}$.

Figure 13 shows a heat map of the spatial distribution of irradiance (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) across each of the treatments (T1 to T6) applied within the cultivation system. Each square represents the position of a plant within the experimental area, and the numerical value indicates the light intensity measured at that point. The colors range from blue (low intensity) to dark red (high intensity), according to the scale located on the right side of the image. Most positions exhibit levels above $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, suggesting an overall homogeneous light distribution, with slight variations observed at the edges and central zones in some treatments. The deep blue areas indicate regions without direct light exposure, corresponding to aisles or gaps between cultivation modules.

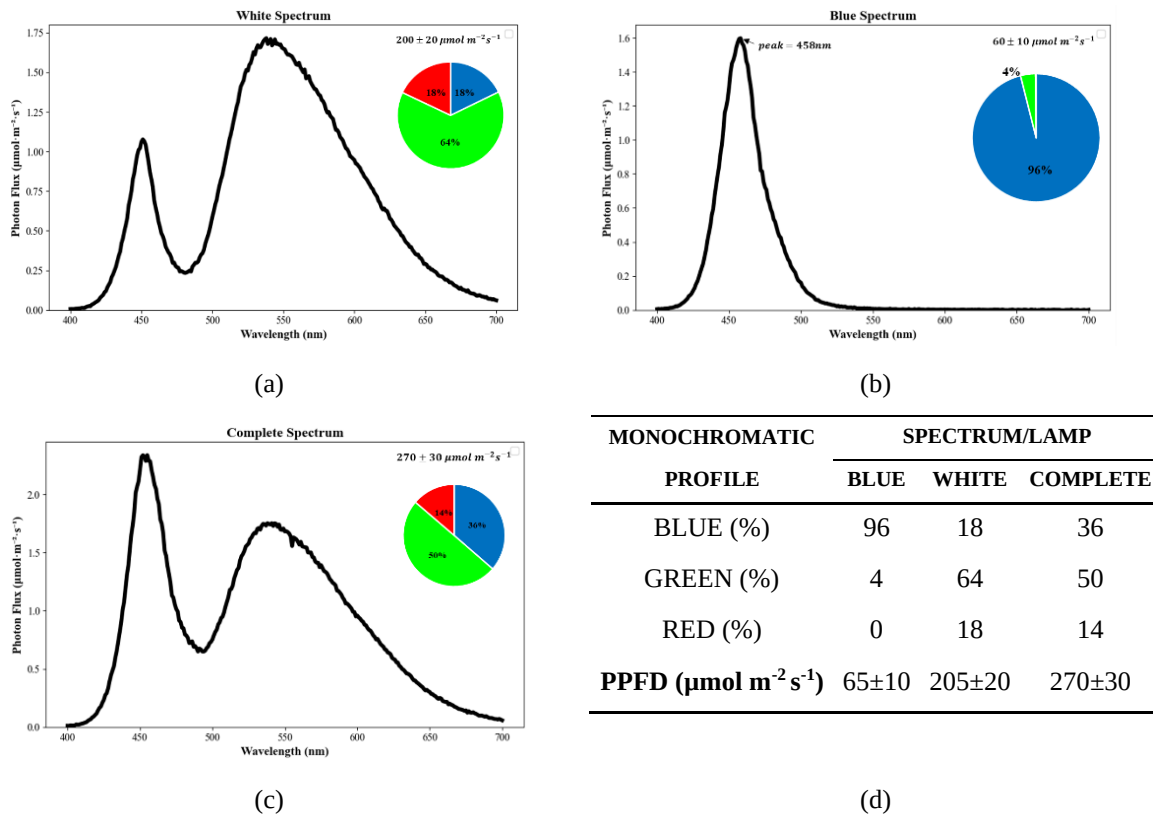


Figure 12. Distribution of Photosynthetic Photon Flux Density (PPFD) across different lighting treatments: (a) White LED lamps; (b) Blue LED lamps; (c) Both light sources combined; (d) Summary of spectral composition and proportions of light recipes for each spectrum

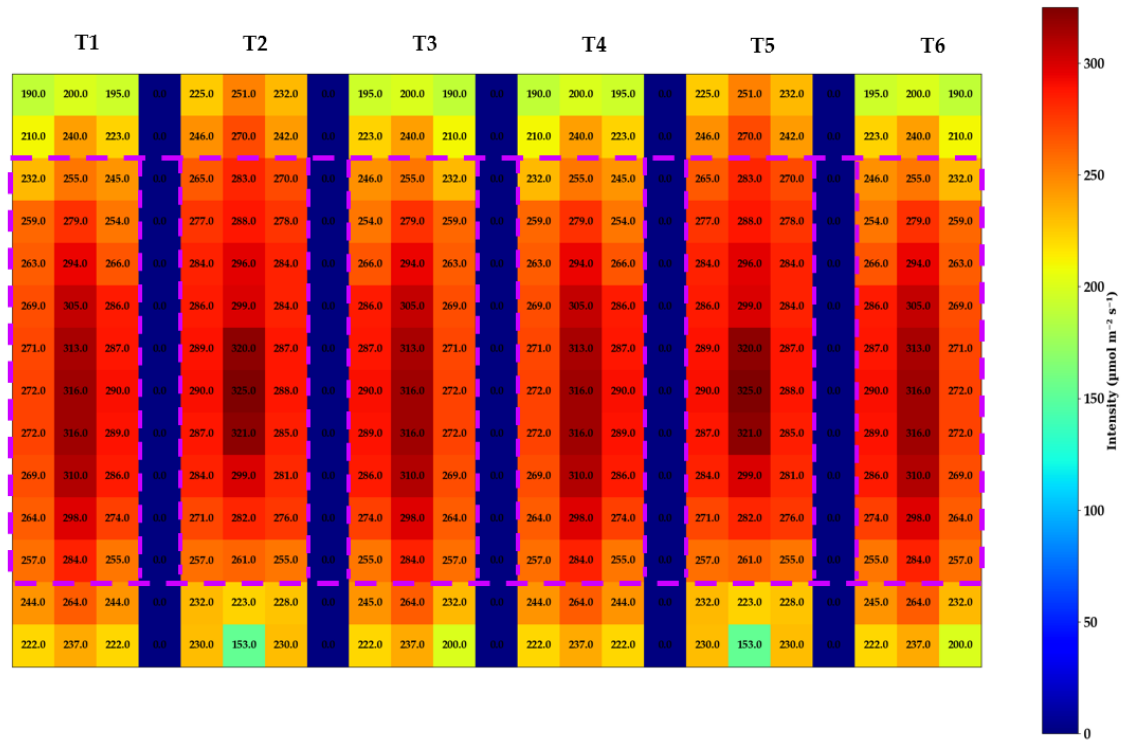


Figure 13. The spatial distribution and uniformity of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) across all treatments (T1–T6) within the cultivation system.

3.4.2 Irrigation System

The automated irrigation system operated with a regulated flow rate between 1 and 2 liters per minute, using submersible water pumps (Kulife, USA) connected to a central control system. The pumps were activated in scheduled cycles of four hours of continuous operation, followed by 20-minute rest intervals. This configuration ensured constant and controlled circulation of the nutrient solution within the NFT system, guaranteeing homogeneous distribution across all cultivation units.

3.4.2 Connections and Monitoring

The entire biomechatronic system was interconnected through an ESP32 CH340C microcontroller (Espressif Systems, China), which communicated with a central Raspberry Pi 4 Model B unit (Raspberry Pi Foundation, United Kingdom) via a local Wi-Fi network. This architecture enabled real-time data collection from distributed sensors, as well as visualization and analysis using the Grafana platform (Grafana Labs, USA). Additionally, two ESP32-CAM units (Espressif Systems, China) were integrated into the Raspberry Pi, allowing remote visual monitoring of the system and crop status throughout the experimental period.

3.5 MEASUREMENTS OF PLANT GROWTH PARAMETERS

3.5.1. Leaf Area (LA) and Number of Leaves (NL)

Leaf area (LA) was determined through digital image analysis utilizing ImageJ software version 1.54g. On day 30 of the treatment, the leaf blades from selected plants were separated and photographed. Images were captured using an Apple iPhone 14 Pro equipped with a 48 MP wide-angle camera (f/1.78 aperture, Sony sensor; Apple Inc., USA), at a distance of 1 meter. Additionally, the leaves were categorized into three groups: Small, Medium, and Large, based on the largest and smallest leaf areas observed across all treatments.

3.5.2. Aerial Fresh and Dry Weight (AFW, ADW)

The Aerial Fresh Weight (AFW) was measured by weighing the root and stem separately using an OHAUS Scout® Balance. After weighing, these samples were placed in paper bags and dried in a forced convection oven (BINDER Drying Oven FD53) at 70°C for 72 hours. Once dried, the samples were weighed again.

3.5.3. Elemental Nutrient Analysis

For the analysis of plant tissue nutrients, samples were dried, packaged in kraft paper bags, and sent to Fertilab Agricultural Laboratory located in Cd. Industrial de Celaya, Guanajuato, Mexico 38010. The laboratory is accredited as “Laboratorio de Ensayo EMA (SA-1359-044/21).” The analysis included the determination of total nitrogen using the Dumas method (accredited). Additionally, the elemental concentrations of boron (B), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), and nickel (Ni) were measured through acid digestion followed by ICP-OES analysis.

3.5.4. Specific Leaf Mass (SLM) and Specific Leaf Area (SLA)

Equation 1 was used to calculate Specific Leaf Mass (SLM), based on leaf dry mass and leaf area, following the method described by Mickens et al.

$$SLM = M_{dr}/LA \quad (1)$$

SLM stands for specific leaf mass per area (mg/cm²), M_{dr} indicates the aerial dry weight (mg), and LA represents leaf area (cm²).

3.5.5. Water Use Efficiency (WUE)

Equation 2 presents the calculation of Water Use Efficiency (WUE), a key indicator of resource utilization under controlled environmental conditions. WUE was determined by dividing the total Plant Fresh Weight (g FW) by the volume of water used (L H₂O) during the crop cycle:

$$WUE = \frac{\text{Plant Fresh Weight (g FW)}}{\text{Water Volume Used (L H}_2\text{O)} - \frac{\text{COLLECTED WATER (L H}_2\text{O)}}{6}} \quad (2)$$

3.5.6 Chlorophyll, EUE and LUE Measurements

Chlorophyll content was evaluated weekly through readings taken at three specific moments of the photoperiod: one hour before the beginning of the light cycle, halfway through the photoperiod, and one hour before its end. Measurements were made using a portable SPAD-502 meter (Minolta, Japan), referencing the middle third of the leaf blade, following the methodology described by Reis et al. (2006). Chlorophyll values were expressed in SPAD units, enabling non-destructive evaluation of relative pigment concentration variations throughout the experiment.

Energy Use Efficiency (EUE) was calculated following the methodology proposed by Pennisi et al. (2020), considering the total fresh weight (FW) of leaves obtained at the end of the experiment. The total electrical consumption of the lighting system was recorded, expressed in kilowatt-hours (kWh). This indicator allowed quantification of system efficiency in terms of fresh biomass produced per unit of energy consumed. The EUE value was determined according to Equation 3.

$$EUE = \frac{\text{Final Leaf Fresh Weight (g FW)}}{\text{Electricity Consumption (kWh)}} \quad (3)$$

Light Use Efficiency (LUE) was calculated based on the definition established by Pennisi et al. (2020), considering the efficiency of converting absorbed photosynthetically active radiation (APAR) into accumulated dry biomass (Dry Biomass, DB). Unlike classical approaches based on Gross Primary Productivity (GPP), this formulation focused on agronomic metrics applicable to performance evaluation in controlled-environment cultivation systems. This parameter assessed physiological crop efficiency as a function of absorbed radiation and dry biomass production during the experiment. Equation 4 represents this relationship.

$$LUE = \frac{\text{Dry Biomass (g m}^{-2}\text{)}}{\text{APAR (MJ m}^{-2}\text{)}} \quad (4)$$

3.5.7 Photosynthetic Efficiency Evaluation (ϕ PSII)

The photosynthetic efficiency of Photosystem II (ϕ PSII) was evaluated weekly using a JUNIOR-PAM chlorophyll fluorometer (Walz, Germany) (figure 14). Measurements were taken from fully expanded leaves selected from the middle third of each plant using an open leaf clip placed over the leaf blade. Two light-adapted and three dark-adapted measurements were performed at different times during the photoperiod.

For light-adapted measurements, the clip remained open to allow ambient illumination, and steady-state fluorescence (F_s) was recorded for at least one minute. Then, a saturating light pulse was applied to obtain the maximum fluorescence under light (F_m'). Subsequently, without removing the clip, the sample was completely covered and kept in darkness for 10 minutes to allow photosynthetic apparatus relaxation. Under these dark conditions, minimum fluorescence (F_o) and maximum fluorescence (F_m) were recorded using a second saturating pulse. Measurements were taken at 8 and 16 hours after the beginning of the daily light interval (DLI) for light- and dark-adapted protocols. An additional dark-adapted measurement was conducted two hours before the start of the photoperiod (-2h DLI) to capture the basal physiological state prior to light exposure.

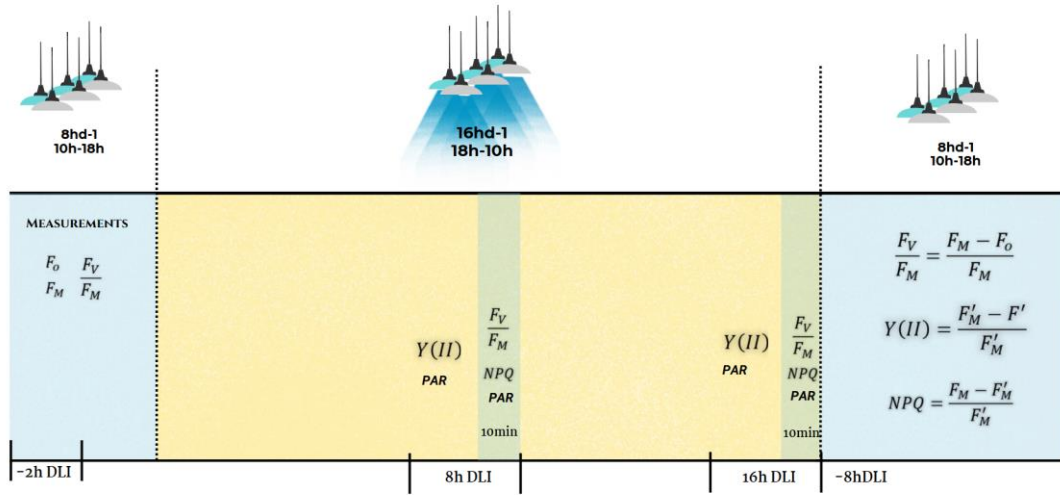


Figure 14. Workflow for the measurement of effective quantum yield [$Y(II)$], maximum quantum efficiency (F_v/F_m), and non-photochemical quenching (NPQ) of PSII in *Lactuca sativa*.

Three key photosynthetic efficiency parameters were calculated from the data: Effective quantum yield of PSII [$Y(II)$], calculated as $(F_m' - F_s)/F_m'$; Maximum quantum yield of PSII (F_v/F_m), calculated as $(F_m - F_o)/F_m$; Non-photochemical quenching (NPQ), calculated as $F_m - F_m'/F_m'$. These indicators reflect the functional status of the photosynthetic apparatus and the physiological responses of plant species to different nutritional and light treatments.

3.6 ENERGY CONSUMPTION

Electrical consumption for each treatment was recorded using a STEREN HER-432 meter (Mexico), operating at 120 V AC and 60 Hz, with a measurement range of 1 mA to 15 A. The analysis considered four main categories: lighting (white and blue lamps), irrigation (submersible pumps), environmental control (Drummond MSDFE12HP-3, BOHN ADT130UC, INKBIRD IHC-200, extractor, intractor, and fans), and automation (Raspberry Pi 4B, ESP32, ESP32-CAM, sensors, actuators, router, status monitor, and regulated power supply). The CO₂ enrichment system (DANOPLUS AZ-7530 controller and generator) was also included. This classification allowed quantification of specific consumption by subsystem within the automated cultivation environment

3.7 STATICAL ANALYSIS

The effect of the association between *Medicago sativa* (MS) and *Lactuca sativa* (LS) under different nitrate concentrations was evaluated through analysis of variance (ANOVA). The experimental design included five association treatments (0%, 33%, 66%, 100%, and 133% NO₃⁻), each with five LS and five MS plants, plus a control group consisting of ten LS monoculture plants, totaling 180 plants (105 LS and 75 MS). To identify significant differences ($p < 0.05$) among treatments for physiological and growth variables, ANOVA was followed by Fisher's Least Significant Difference (LSD) test for mean comparisons. Additionally, correlation and regression analyses were conducted to determine relationships between growth, nutrition, and photosynthetic efficiency variables. All statistical analyses were performed using MINITAB 20 software, with a confidence level of 95% ($\alpha = 0.05$).

IV. RESULTS.

4.1 EVALUATION OF ENVIRONMENTAL CONDITIONS AND CROP RESPONSE

The biomechatronic system maintained a stable environmental setting that favored the development of *Lactuca sativa* and *Medicago sativa*. Thermal regulation remained within the physiologically optimal range, averaging 20.54 ± 1.12 °C, thus avoiding fluctuations that could affect metabolic processes. Simultaneously, relative humidity was controlled at $57.67 \pm 4.12\%$, promoting efficient transpiration and proper tissue hydration. Additionally, CO₂ concentration stabilized at 1078.56 ± 129.56 ppm, ensuring continuous availability for photosynthesis without reaching saturation levels that could compromise efficiency.

Light distribution was homogeneous, with variations below 10% across positions, ensuring uniform irradiance over the plant canopy and minimizing differential radiation use among plants. Irrigation supply was delivered at a constant flow of 1.8 to 2.0 L/min, in cycles of four hours of continuous operation followed by 20-minute resting intervals, maintaining adequate water availability without causing root zone flooding. The NFT system's inclination, adjusted between 1.5% and 2%, promoted a continuous laminar flow of the nutrient solution, improving root oxygenation and preventing localized accumulations.

4.2 NUTRITIVE SOLUTION DYNAMICS

4.2.1 Water Consumption

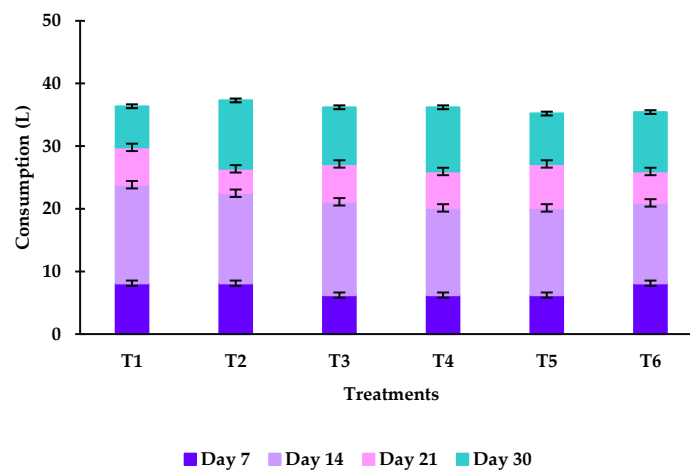


Figure 15. Weekly water consumption per treatment throughout the four-week experimental period.

Figure 15 illustrates the water consumption patterns across treatments during the four-week cultivation period. Treatments T1 and T2 demonstrated similar total water use, averaging 39.4 L each. In contrast, T3, T4, and T5 showed slightly higher water retention, averaging 41.3 L. Among all treatments, T1 exhibited the highest overall consumption, reducing its water volume to 23.7 L, while T4 and T5 retained the most water, both ending at 27.4 L. By the end of the experiment, water levels in T1 and T2 were the lowest, with 11.1 L and 10.2 L, respectively, compared to the control (T0) which remained at 12.1 L. Notably, T2 experienced the sharpest decline in water availability, while T5 retained the highest final volume, indicating a potential dose-dependent effect related to treatment application.

4.2.2 pH dynamics

The pH dynamics across the treatments, as shown in Table 3, exhibited fluctuations over the four-week period, with adjustments made to maintain optimal ranges (5.5–6.5 for hydroponic low salinity and medium salinity). The T6 group consistently acidified the nutrient solution by the second, third, and fourth weeks. In contrast, sharp increases in pH were observed in treatments T1 (6.87), T2 (7.36), T3 (6.92), and T5 (7.15). Treatment T4 experienced a smaller increase in pH, reaching 6.25. The alfalfa treatments stabilized between 6.0 and 6.5, except for T2, which remained slightly elevated at 6.46. Notably, treatment T4 required minimal adjustments.

Table 3. Variation in nutrient solution pH across treatments over the course of four weeks (W).

Treatments	W0 (Day 0)	W1		W2		W3		W4(Day 30)
		Initial	Final	Initial	Final	Initial	Final	
T1	6.02 ^a	6.87 ^c	5.99 ^a	6.88 ^c	6.02 ^a	6.21 ^c	6.16 ^a	6.06 ^b
T2	6 ^a	7.36 ^a	5.94 ^a	7.33 ^a	6.06 ^a	6.85 ^a	6.06 ^b	6.46 ^a
T3	6.01 ^a	6.92 ^c	5.98 ^a	6.99 ^b	6.03 ^a	6.85 ^a	6.01 ^b	6.13 ^b
T4	5.99 ^a	6.25 ^d	6.04 ^a	6.24 ^d	6.09 ^a	6.25 ^c	6.06 ^b	6.09 ^b
T5	6.01 ^a	7.15 ^b	6 ^a	6.9 ^c	6.04 ^a	6.59 ^b	6.03 ^b	6.13 ^b
T6	6 ^a	6.04 ^e	6.04 ^a	5.37 ^e	5.9 ^a	5.5 ^d	5.9 ^b	5.3 ^c

4.2.3 Electrical Conductivity (EC)

Figure 16 shows that the electrical conductivity (EC) values gradually increased across all treatments, indicating the accumulation of ions as water was consumed. By the fourth week of the experiment, the control group exhibited the highest EC at 2.20 dS m⁻¹. In comparison, Treatments T1 and T2 recorded lower final EC values of 2.04 dS m⁻¹, which aligned with their higher water consumption (as mentioned in Section 1.1). Treatments T4 and T5 reached an EC of 2.06 dS m⁻¹, suggesting effective nutrient uptake. Despite these increasing trends, the

EC levels remained within the expected range throughout the experiment, eliminating the need for adjustments and ensuring stable nutrient availability.

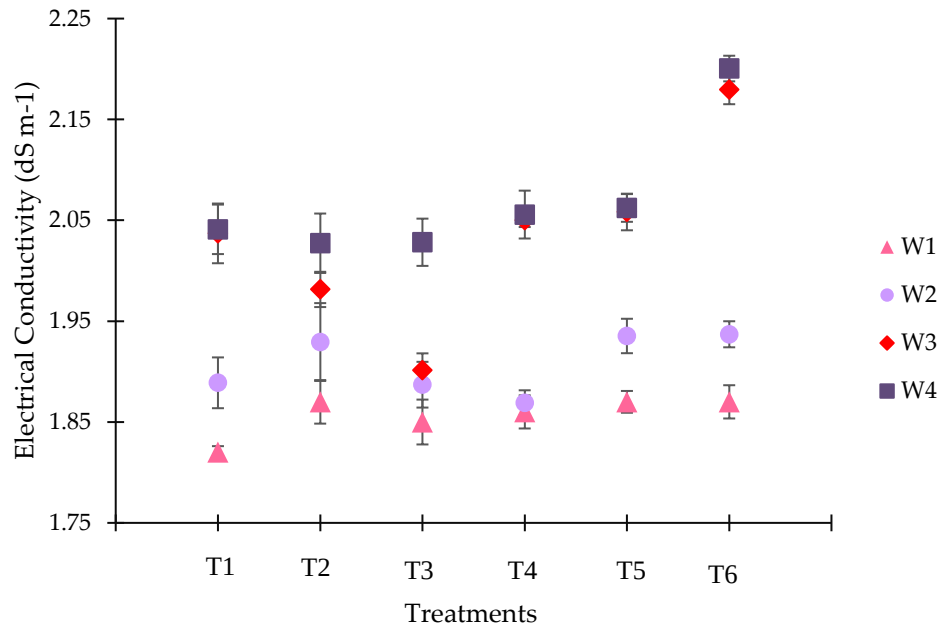


Figure 16. EC of the nutrient solution by treatment over the four-week experimental period.

4.3 PLANT GROWTH AND BIOMASS ACCUMULATION.

Table 4 presents the data on fresh and dry biomass accumulation across treatments. An increase in both fresh and dry mass was observed with higher levels of nitrogen fertilization. The total fresh weight of the plants increased notably, rising from 37.21 g in treatment T1 to 136.75 g in treatment T5, with the control group exhibiting the highest weight at 147.83 g. Notably, the leaf fresh weight in treatments with higher nitrogen levels reached up to 92.9% in T5 compared to the control, whereas T1 had the lowest fresh weight percentage (77.9%) and a comparatively higher dry weight per-centage (22.1%). This pattern suggests that increased nitrogen availability promotes greater water accumulation in plant tissues. Additionally, the mass lost during drying followed the same trend, being significantly lower in T1 (12.84 g) and highest in the control group (104.71 g), further indicating enhanced tissue hydration due to nitrogen fertilization.

Table 4. Effect of treatments on leaf area, specific leaf mass (SLM), leaf number, fresh mass, dry mass, root biomass, and water use efficiency (WUE) in lettuce plants at 30 days after transplanting.

Plant Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
						
Leaf number	16.50 ^b	29.00 ^a	30.33 ^a	29.00 ^a	24.83 ^b	28.67 ^{ab}
Leaf area (cm ²)	344.90 ^c	1297.14 ^{ab}	1512.62 ^a	1041.95 ^b	1173.23 ^b	1357.75 ^{ab}
Fresh mass (g)	17.93 ^c	88.30 ^{ab}	104.57 ^{ab}	83.55 ^b	98.79 ^{ab}	114.60 ^a
Dry mass (g)	5.09 ^b	7.63 ^{ab}	8.95 ^a	7.63 ^{ab}	7.55 ^{ab}	9.90 ^a
Root mass (g)	19.27 ^c	28.58 ^b	33.93 ^{ab}	34.90 ^{ab}	37.97 ^a	33.23 ^{ab}
SLM (mg cm ⁻²)	15.23 ^a	6.02 ^b	5.91 ^b	7.363 ^b	6.48 ^b	7.33 ^b
WUE (gDW L ⁻¹)	1.572 ^b	2.242 ^{ab}	2.811 ^a	2.395 ^{ab}	2.522 ^{ab}	3.264 ^a

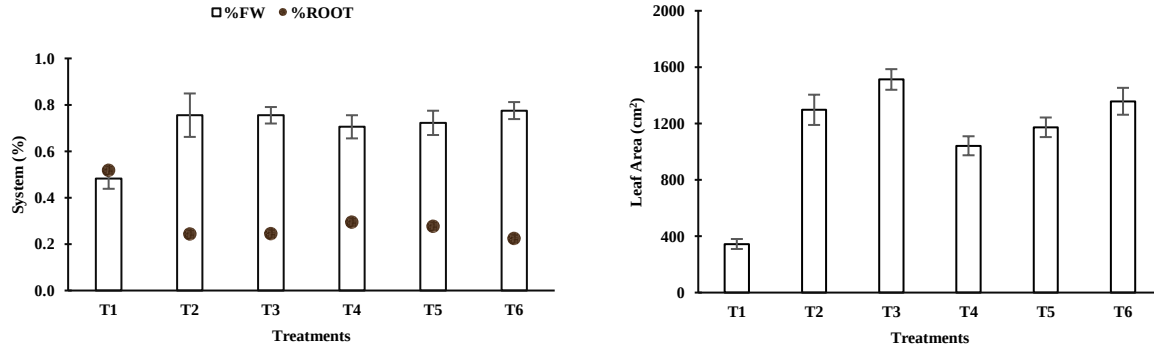


Figure 17. Effects of nitrate availability on biomass allocation and leaf development in lettuce. (a) Proportion of fresh weight (%FW) versus root weight (%Root) across treatments. (b) Leaf area variation per treatment at 30 days after transplanting.

Figure 17 depicts the changes in root mass and the proportion of root biomass across different treatments. The root mass showed a consistent upward trend, increasing from 19.27 g in treatment T1 to 37.97 g in T5, with T6 recording a root mass of 33.23 g. This trend indicates an enhanced ability for water and nutrient uptake when nitrate (NO_3^-) availability is higher. However, the percentage of root biomass (% root) relative to the total plant mass decreased as NO_3^- levels increased—falling from 52 % in T1 to 28 % in T5 and reaching a low of 22 % in T6. This decline suggests that, under low nitrogen availability conditions, plants invest a greater proportion of their

biomass in root development to maximize nutrient acquisition. In contrast, higher nitrate levels promote a shift in biomass allocation toward the shoots, which increases the proportion of aboveground fresh weight (% FW).

In treatment T1, plants displayed the lowest number of leaves, averaging 16.50, while treatment T3 had the highest count at 30.33 leaves. Treatment T6 followed closely with 28.67 leaves, and T2 had 29.00 leaves. The total leaf area significantly increased with the application of nitrate. T1 had a leaf area of 344.90 cm², whereas the nitrate treatments ranged from 1,041.95 cm² in T4 to 1,512.62 cm² in T3. The control group reached a leaf area of 1,357.75 cm². Leaves from T1 exhibited a significantly higher specific leaf mass (SLM) of 14.75 mg/cm², indicating that they were denser and thicker, likely due to nitrate deficiency. In contrast, the nitrogen fertilization treatments showed an SLM ranging from 5.88 to 7.32 mg/cm², which suggests that these leaves were thinner and broader, optimizing light capture. WUE was significantly lower in T1, at 1.572 g dry weight (DW) per liter of water (H₂O), compared to the fertilized treatments. T6 attained the highest efficiency at 3.264 g DW per liter of H₂O, followed by T3 with 2.811 g DW per liter of H₂O.

4.4 ACCUMULATION OF MINERAL ELEMENTS IN PLANT TISSUE.

Table 5. Macronutrient and micronutrient accumulation in lettuce plants harvested 30 days after transplanting. Values represent means per treatment. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Tukey's multiple comparison test.

NUTRIENTS		Treatments						Literature Ranges		
(mg g ⁻¹ DW)		T1	T2	T3	T4	T5	T6	min	max	References
Macronutrients	Nitrogen (N)	12.8 ^c	40.4 ^{ab}	38.6 ^b	43.8 ^a	41.3 ^{ab}	40.4 ^{ab}	24	40	(Martínez-Moreno et al., 2024; Uchibayashi et al., 2024; Waterland et al., 2017)
	Phosphorus (P)	3.8 ^d	10.1 ^b	8.6 ^c	10.9 ^{ab}	11.3 ^a	11.2 ^a	6	9.5	(Levine & Mattson, 2021; Matraszek, 2015; Sardar et al., 2023)
	Potassium (K)	37.3 ^c	85.2 ^a	77.2 ^b	79 ^{ab}	82.6 ^{ab}	77.6 ^b	55	85	(Matraszek, 2015; Sugiarto et al., 2024)
	Calcium (Ca)	6.3 ^c	13.2 ^a	11.7 ^b	11.1 ^b	11.3 ^b	11.8 ^b	9	14	(Faran et al., 2023; Qiao et al., 2023)
	Magnesium (Mg)	2 ^c	3.8 ^a	3.3 ^b	3.7 ^a	3.7 ^a	3.1 ^b	2.5	4	(Souza et al., 2017; Thakur et al., 2023)

Micronutrients	Sulfur (S)	1.5 ^d	2.6^{bc}	2.4 ^c	3.0^a	2.9^a	2.8^{ab}	1.8	2.6	(Avendaño-Abarca et al., 2020; Waterland et al., 2017)
	Iron (Fe)	19.5 ^d	39.1^b	34.8 ^c	39.2^b	42^b	48.3^a	24	35	(Buturi et al., 2022; Dasgan et al., 2023; Thakur et al., 2023)
	Zinc (Zn)	65.3 ^c	241^{ab}	262^a	244^{ab}	223^b	234^b	120	190	(Mohammadi & Khoshgoftarmanesh, 2014; Rengel et al., 2022)
	Manganese (Mn)	239 ^d	374 ^c	477^a	419 ^b	373 ^c	367 ^c	310	450	(Dasgan et al., 2023; Waterland et al., 2017)
	Copper (Cu)	3.16 ^d	9.97 ^c	9.63 ^c	11.5^b	11.2^b	12.9^a	6	10	(Dasgan et al., 2023; Waterland et al., 2017)
	Boron (B)	30.6 ^d	42.5^a	36.4 ^b	42.3^a	34.8 ^{bc}	31.5 ^{cd}	28	40	(Bhatla & Lal, 2023; Dasgan et al., 2023)
	Nickel (Ni)	5.52^a	0.37 ^c	0.16 ^c	0.22 ^c	0.2 ^c	0.64^b	0.15	0.35	(Khoshgoftarmanesh et al., 2011; Mohammadi & Khoshgoftarmanesh, 2014; J. Zhao et al., 2019)
	Molybdenum (Mo)	0.1^b	0.12^a	0.06 ^d	0.05 ^e	0.09 ^c	0.1^b	0.04	0.09	(Dasgan et al., 2023; Moncada et al., 2018)

Table 5 summarizes the macronutrient content across treatments. A significant difference was observed in treatment T1, where levels of calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), sulfur (S), and nitrogen (N) were all notably lower per unit of dry biomass compared to other treatments. In contrast, the remaining treatments displayed macronutrient concentrations comparable to the control group. Nitrogen (N) content exhibited clear statistical differences. The lowest value was recorded in T1 (12.8c), while fertilized treatments reached significantly higher values, peaking in T4 (43.8a). Treatments T5 (41.3ab) and T6 (40.4ab) did not differ significantly from each other, suggesting that nitrogen uptake may plateau beyond a certain threshold of availability. Similarly, phosphorus (P) content varied significantly across treatments. T1 had the lowest value (3.8d), while the highest concentrations were found in T5 (11.3a) and T6 (11.2a). Intermediate values were observed in T2 (10.1b) and T3 (8.6c), indicating that P uptake improves with nitrogen availability, up to a saturation point.

Potassium (K) showed a significant response to fertilization, increasing from 37.3 mg kg⁻¹ at T1 to a maximum of 85.2 mg kg⁻¹ at T2. However, the higher treatments (T3, T4, and T5) displayed lower potassium levels, measuring 77.2 mg kg⁻¹, 79.0 mg kg⁻¹, and 82.6 mg kg⁻¹, respectively. Treatment T6 also had a similar value of 77.6 mg kg⁻¹. These results indicate that potassium uptake is more efficient at moderate levels of nitrogen fertilization, and no significant increases in potassium are observed at higher dosages. Calcium (Ca) recorded its lowest value at T1, with a concentration of 6.3 mg kg⁻¹, and reached its peak at T2 with 13.2 mg kg⁻¹. The subsequent treatments with fertilization showed similar calcium values ranging from 11.1 mg kg⁻¹ to 11.8 mg kg⁻¹, with no significant differences between them. This suggests that calcium uptake stabilizes after reaching a certain level of available nitrogen.

Magnesium (Mg) demonstrated a similar trend, with the lowest value recorded at T1 (2c) and the highest values observed at T2 and T4 (3.8a and 3.7a, respectively). The values in the other treatments were intermediate, measuring 3.3b at T3 and 3.1b at T6. This pattern suggests that nitrogen availability enhances magnesium uptake to some extent.

In terms of sulfur (S), the lowest value was also noted at T1 (1.5d), which increased with fertilization, reaching its peak at T4 (3a). Although the values at T5 and in the control treatment were somewhat lower (2.9a and 2.8ab, respectively), there was no significant difference from T4. This indicates that sulfur responds positively to nitrogen up to a certain level.

Micronutrient levels varied with different nitrogen fertilization treatments, showing significant statistical differences among them. Iron (Fe) concentrations were lowest in the T1 treatment, at 19.5d, but increased with nitrogen fertilization, reaching the highest level in the control group at 48.3a. The T2, T4, and T5 treatments displayed intermediate iron values of 39.1b, 39.2b, and 42b, respectively. There were no significant differences among these three treatments, suggesting that while nitrogen availability enhances iron uptake, other factors may limit its absorption beyond a certain point.

Zinc (Zn) exhibited its lowest concentration at T1 (65.3c) and reached its peak at T4 (262a). The zinc levels for T2 and T4 were similar, recorded at 241ab and 244ab, respectively. In contrast, T5 and the control group showed lower levels at 223b and 234b. This indicates that zinc uptake is higher at intermediate fertilization levels but tends to decrease at elevated levels.

Manganese (Mn) had its lowest concentration at T1 (239d) and peaked at T3 (477a). T4 displayed an intermediate value of 419b, while treatments T2, T5, and T6 fell within a similar range of 373c to 374c. This suggests that nitrogen availability enhances manganese uptake up to T3, but beyond that, the uptake declines.

Copper (Cu) exhibited a pattern similar to that of other nutrients, showing its lowest value at T1 (3.16d) and peaking at T6 (12.9a). Treatments T4 and T5 displayed intermediate values of 11.5b and 11.2b, respectively,

while T2 and T3 had significantly lower values at 9.97c and 9.63c. These results suggest that copper uptake improves with fertilization; however, the control group exhibited even higher uptake, potentially due to other environmental factors.

Boron (B) reached its highest level at T2 (42.5a), with similarly high values observed at T4 (42.3a). In contrast, T5 showed a significant decrease (34.8bc), and T1 recorded the lowest value (30.6d). This indicates that boron uptake is optimal at moderate levels of nitrogen fertilization but tends to decline when nitrogen is in excess.

Nickel (Ni) exhibited a pattern that contrasts with most other nutrients. It reached its highest value at T1 (5.52a) but experienced a significant decline in the fertilization treatments, dropping to its lowest level at T3 (0.16c). Although T6 recorded a higher value (0.64b), it was still significantly lower than T1. This suggests that the availability of nitrogen decreases nickel uptake, likely because the presence of sufficient available nitrogen reduces the need for this element.

In contrast, molybdenum (Mo) showed its highest uptake at T2 (0.12a), while T4 registered the lowest value (0.05e). The other treatments displayed intermediate values, with T6 at 0.1b and T5 at 0.09c. This indicates that molybdenum uptake is negatively impacted by high levels of nitrogen fertilization, possibly due to competition with other anions in the nutrient solution.

4.5 CHLOROPHYLL ACCUMULATION DYNAMICS IN *LACTUCA SATIVA*

The evolution of chlorophyll content, expressed in SPAD units, showed significant differences between treatments and evaluation times (Table 6). At the beginning of the experiment (day 0), no differences between treatments were observed, registering a uniform value of 17 ± 0.00 SPAD in all groups. During the first week, treatments T1, T3 and T5 presented significantly higher values ($p < 0.05$) compared to T2, T4 and T6, suggesting a differentiated early response to fertilization or plant association treatments. In the second week, a generalized increase in chlorophyll content was observed in all treatments, with no significant differences detected among them. From the third week on, clear differences emerged: treatment T6 recorded the highest value (41.01 ± 8.52 SPAD), followed by T3, T5 and T4, which showed intermediate values. In the fourth week, treatments T3, T4, T5 and T6 maintained the highest chlorophyll contents, significantly exceeding treatments T1 and T2. At the end of the experiment, the highest chlorophyll content was recorded in T5 (53.20 ± 9.66 SPAD), followed by T3 (51.16 ± 4.18 SPAD), T4 (50.88 ± 4.29 SPAD) and T6 (50.07 ± 2.51 SPAD). In contrast, T1 and T2 presented the lowest values, which could be associated with limitations in nitrogen availability or the absence of symbiotic interaction.

Table 6. Chlorophyll content (SPAD units) measured weekly in *Lactuca sativa* under different nitrate treatments. Values represent mean $\pm$ standard deviation. Different letters within the same column indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$).

Treatments	SPAD units				
	Day 0	W1	W2	W3	W4
T1	17 $\pm$ 0.00 ^a	20.67 $\pm$ 0.51 ^a	28.12 $\pm$ 4.47 ^a	29.85 $\pm$ 2.25 ^c	37.16 $\pm$ 2.47 ^b
T2	17 $\pm$ 0.00 ^a	17.23 $\pm$ 1.94 ^b	27.45 $\pm$ 3.88 ^a	33.92 $\pm$ 3.40 ^{bc}	38.30 $\pm$ 2.19 ^b
T3	17 $\pm$ 0.00 ^a	21.07 $\pm$ 0.51 ^a	33.22 $\pm$ 4.40 ^a	37.61 $\pm$ 3.90 ^{ab}	51.16 $\pm$ 4.18 ^a
T4	17 $\pm$ 0.00 ^a	18.10 $\pm$ 0.51 ^b	30.76 $\pm$ 6.73 ^a	35.81 $\pm$ 5.21 ^{ab}	50.88 $\pm$ 4.29 ^a
T5	17 $\pm$ 0.00 ^a	21.67 $\pm$ 0.51 ^a	29.31 $\pm$ 6.75 ^a	37.48 $\pm$ 2.65 ^{ab}	53.20 $\pm$ 9.66 ^a
T6	17 $\pm$ 0.00 ^a	17.27 $\pm$ 1.26 ^b	30.34 $\pm$ 4.98 ^a	41.01 $\pm$ 8.52 ^a	50.07 $\pm$ 2.51 ^a

4.5.1 Light Use Efficiency (LUE) and Electricity Use Efficiency (EUE)

Figure 18a represents the Light Use Efficiency (LUE) values presented remarkable differences between treatments. The treatment with 66 % nitrogen showed the highest efficiency, with a value of 0.82 g DW mol⁻¹, indicating a more effective utilization of photosynthetically active radiation. Interestingly, the control treatment (without inoculation of *Medicago sativa*) reached an even higher value (0.90 g DW mol⁻¹), with no evidence of a reduction attributable to the absence of legume symbiosis. In contrast, the treatment with 0 % nitrogen presented the lowest value (0.46 g DW mol⁻¹), reflecting a clear nutritional limitation that affected photosynthetic efficiency. The 100% and 133% nitrogen treatments did not significantly exceed the 66% nitrogen treatment, suggesting that high nitrate levels do not improve light efficiency, possibly due to saturation or metabolic dilution effects.

Electricity Use Efficiency (EUE) showed marked differences between treatments (Figure 18b). The control treatment registered the highest value (1.50 g FW kWh⁻¹), followed by the 66 % nitrogen treatment (1.37 g FW kWh⁻¹), reflecting a more efficient conversion of the electrical energy consumed into fresh biomass. In contrast, the 0 % nitrogen treatment showed the lowest energy efficiency (0.23 g FW kWh⁻¹), indicating severe limitations in plant growth due to nitrogen deficiency. Similar to what was observed in LUE, the treatments with higher nitrate concentrations (100 % and 133 %) did not show significant improvements with respect to the 66 %

treatment, suggesting the existence of a saturation threshold in the productive response to increases in nitrogen availability.

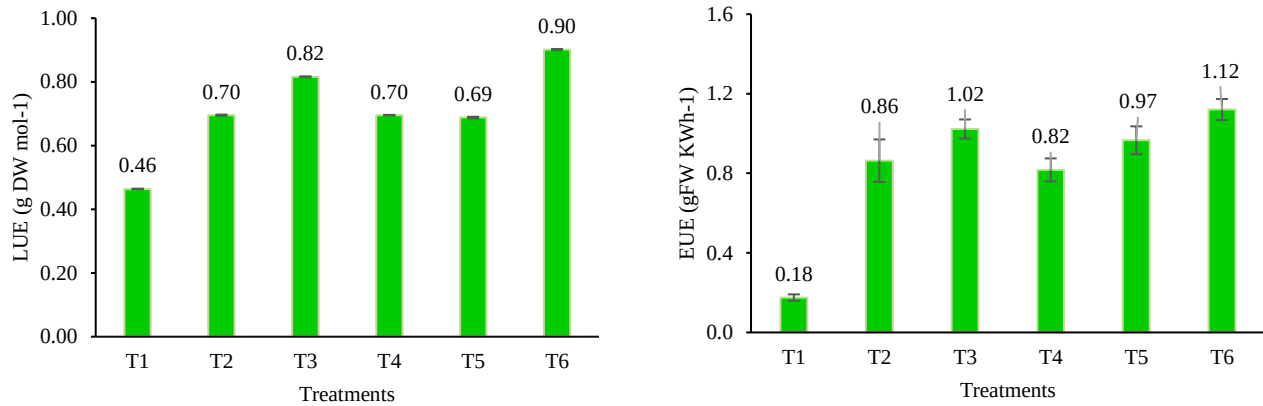


Figure 18. LS LUE and EUE. (a) Light Use Efficiency (g DW mol⁻¹) in *Lactuca sativa* under different nitrate treatments. Error bars represent standard deviation. (b) Energy use efficiency (g FW kWh⁻¹) in *Lactuca sativa* under different nitrate treatments. Error bars represent standard deviation.

4.6 PHOTOSYNTHETIC PERFORMANCE. QUANTUM YIELD OF ϕ PSII [Y(II)]

The quantum efficiency of photosystem II [Y(II)] was evaluated at 8 and 16 h of the exposure period to photosynthetically active radiation for four weeks (Figure 19). In general, the values recorded at 8 h were higher than those measured at 16 h, indicating a progressive reduction of photosynthetic efficiency towards the end of the daily illumination period. In the treatment with 0 % nitrogen (T1), associated with *Medicago sativa*, Y(II) showed a slight decrease over time, going from 0.712 in week 1 to 0.683 in week 4 at 8 h, while at 16 h the values remained relatively stable, between 0.617 and 0.632. The treatment with 33 % N (T2) presented greater stability, with minimal variations between weeks both at 8 h and 16 h. On the other hand, the treatment with 66 % nitrogen (T3) registered the highest Y(II) values, reaching a maximum of 0.747 at 8 h in week 4, and remaining around 0.715 at 16 h, standing out for its high temporal stability. The treatment with 100 % N (T4) showed an increasing trend, going from 0.7020 to 0.7347 at 8 h between week 1 and 4, and from 0.6877 to 0.7098 at 16 h. In contrast, the treatment with 133 % N (T5) evidenced a reduction in efficiency at week 4, registering values of 0.6913 at 8 h and 0.6672 at 16 h. Finally, the control treatment (T6), without association with *Medicago sativa*, maintained relatively constant values throughout the experiment, although with a slight decrease at the end (from 0.6997 at 8 h and 0.6722 at 16 h).

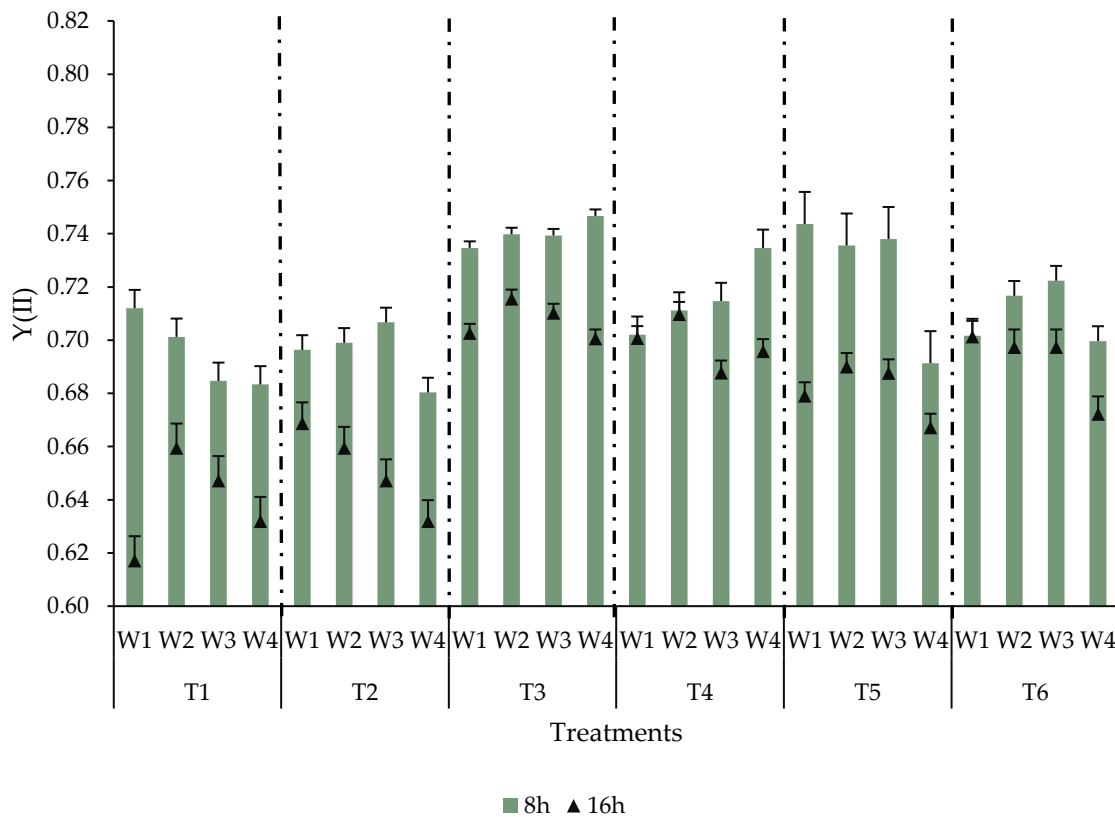


Figure 19. Effective quantum yield of ϕPSII [$Y(\text{II})$] in *Lactuca sativa* under different nitrate treatments, measured at 8 h and 16 h over four weeks. Error bars represent standard deviation.

4.7 PHOTOPROTECTION AND LIGHT STRESS-MAXIMUM QUANTUM EFFICIENCY OF PSII

Figure 20 shows the maximum quantum efficiency of photosystem II [F_v/F_m] was evaluated at -2, 8 and 16 h of the light exposure period for four weeks, under conditions of association with *Medicago sativa*, except in the control treatment. Values obtained two hours before the onset of the photoperiod (-2) remained stable, between 0.75 and 0.79 in most treatments, reflecting an adequate basal efficiency of PSII. However, in week 4, the 0 % N treatment recorded a reduction to 0.7500, indicating a functional weakening of the photosystem in the absence of fertilization. F_v/F_m values evaluated at 8 h, after a brief dark adaptation period, showed clear differences between treatments. The treatment with 66 % nitrogen reached the highest value at week 4 (0.7511), while the treatment with 0 % N presented the lowest value (0.6858), showing a lower photosynthetic recovery capacity under nitrogen restriction. At 16 h, a generalized decrease was observed in all treatments, attributable to accumulated light stress after prolonged exposure to radiation. Despite this reduction, the 66 % N treatment and the control maintained the

highest values at week 4 (0.7256 and 0.7421, respectively), indicating greater functional stability of PSII in the face of stress. In contrast, the 133 % N treatment showed a remarkable drop to 0.6930, suggesting a lower efficiency of photoprotection mechanisms under overfertilization conditions.

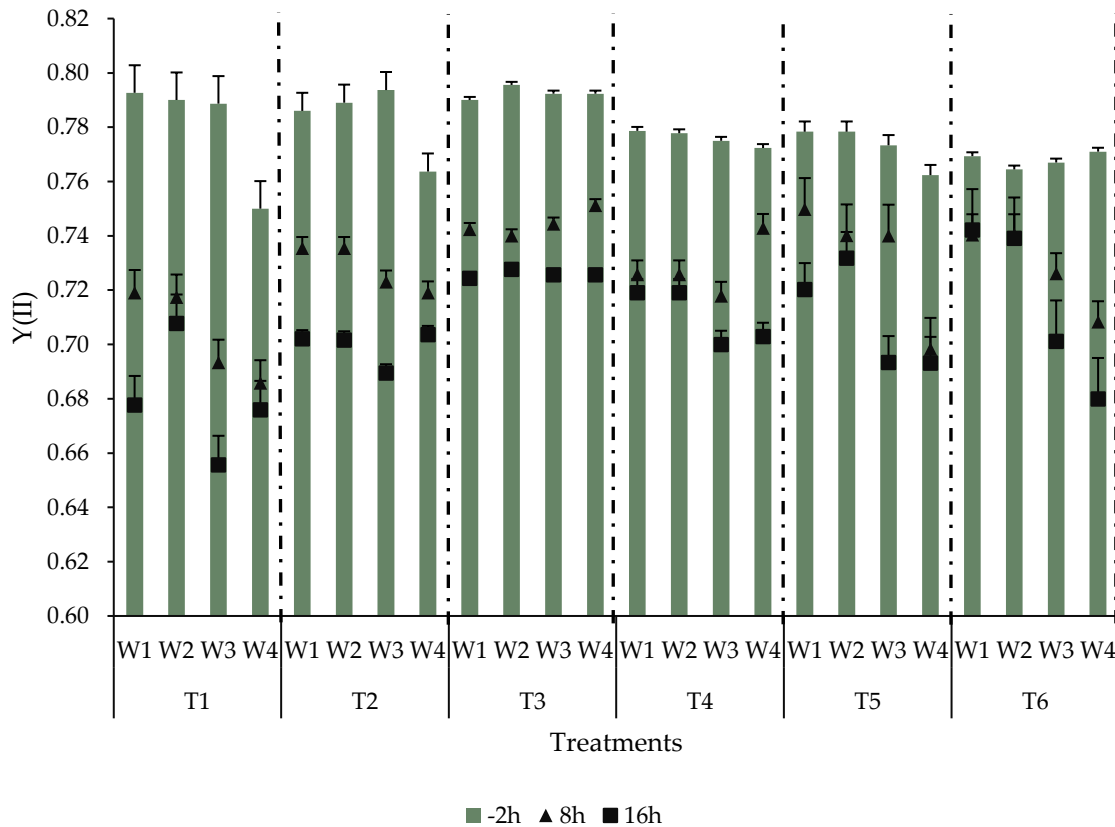


Figure 20. *Fv/Fm* values in *Lactuca sativa* under different nitrate treatments with *Medicago sativa*.

Measurements at -2, 8, and 16 h. Error bars show standard deviation.

Figure 21 shows the non-photochemical dissipation (NPQ) values measured at 8 and 16 hours for four weeks for the different treatments. At 8 hours, variations were observed between treatments and weeks. The 33 % nitrogen treatment (T2) showed the highest value at week 4 (0.568), while the 66 % nitrogen treatment (T3) showed the lowest values, with records below 0.1 in all weeks. At 16 hours, NPQ values increased in most treatments. Treatments 0 % (T1) and 33 % (T2) reached the highest levels, with values up to 0.534 in week 4. In contrast, treatments 66 % (T3) and control (T6) presented lower values, below 0.3 in the same week.

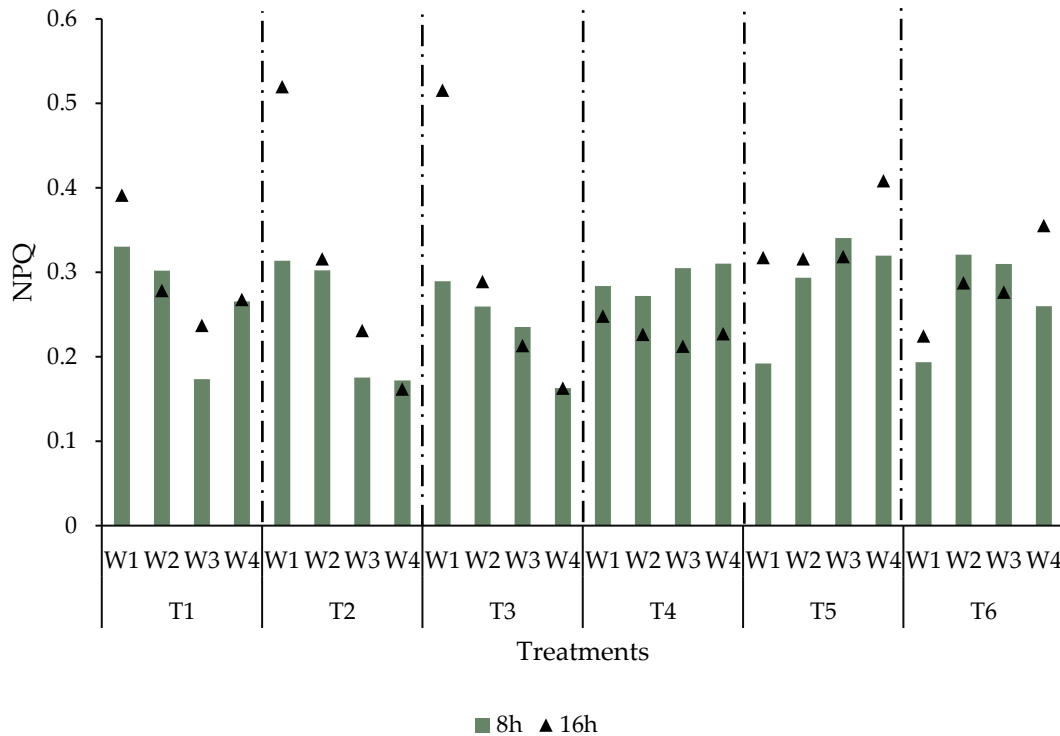


Figure 21. Non-photochemical dissipation (NPQ) at 8 hours (blue bars) and 16 hours (orange dots) during four weeks (W1-W4) in the different treatments.

4.8 ENERGY CONSUMPTION

Figure 22 shows the results of energy consumption by system component during a production cycle. The highest consumption was recorded in the lighting system, with a total of 293.8 kWh, representing the most significant fraction of total energy use. This value reflects the high energy demand associated with the continuous operation of LED lamps during the extended photoperiod. In second place, the temperature control -which included the operation of the minisplit, fans and exhaust fan- presented a consumption of 206.1 kWh per cycle. The irrigation system, composed of submersible pumps and controllers, registered an intermediate consumption of 99.7 kWh, while the general automation system (Raspberry Pi, sensors, relays and ESP32 modules) showed significantly lower consumption (11.3 kWh). Finally, the CO₂ control had the lowest consumption, with only 2.7 kWh. These results show a clear concentration of energy use in the lighting component, followed by climate control and irrigation, while the auxiliary systems operated with high energy efficiency.

Maestría en Ciencias en Ingeniería Mecatrónica
 SYNERGISTIC EFFECTS OF NITRATE UNDER LETTUCE ALFALFA ASSOCIATIONS IN
 BIOMECHATRONIC NFT VERTICAL FARMING

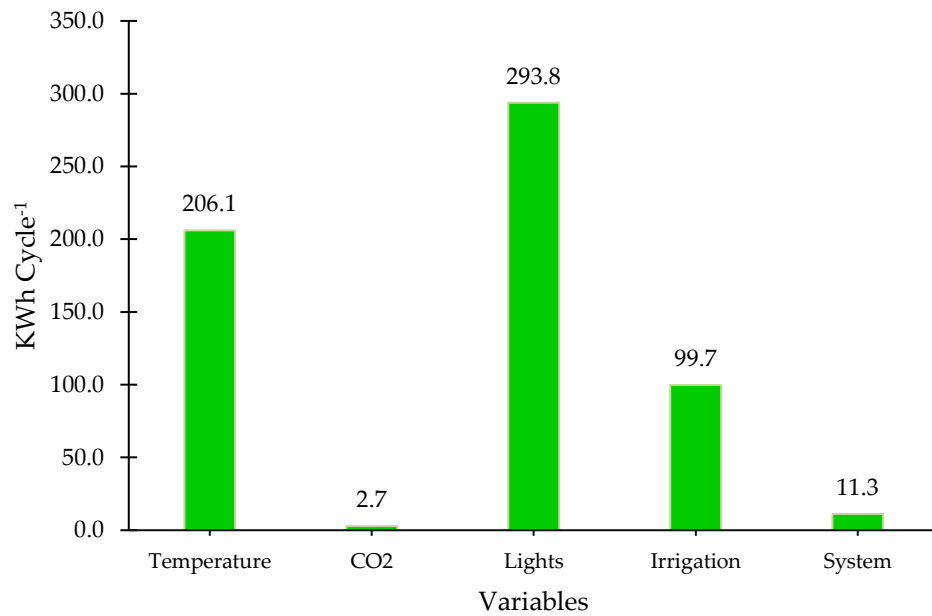


Figure 22. Energy consumption (kWh cycle⁻¹) of the main components of the cultivation system: temperature control, CO₂ enrichment, lighting, irrigation and automation. The data represents the cumulative consumption per variable during a production cycle.

V. DISCUSSION

The results clearly indicate that the availability of nitrate (NO_3^-) in vertical hydroponic nutrient film technique (NFT) systems significantly influences the physiological, nutritional, and morphological development of *Lactuca sativa* (lettuce) when grown alongside *Medicago sativa* (alfalfa). These findings provide quantifiable evidence to address a complex issue: determining the optimal nitrate levels in controlled crop environments while balancing productivity and the nutritional quality of the final product. The presence of *Medicago sativa* not only modulated physicochemical parameters—such as pH (6.1–6.7) and electrical conductivity (EC, which decreased from 2.1 to 1.5 mS cm^{-1})—but also enhanced nutrient absorption and the balance within the rhizosphere. This modulation likely improved ionic availability and nutrient uptake efficiency, as supported by (Liu et al., 2021; X. Zhang et al., 2021).

Moreover, this study demonstrates that the integration of *Medicago sativa* into controlled NFT systems significantly promotes improvements in growth, photosynthetic efficiency, and resource use in *Lactuca sativa*, particularly under moderate nitrogen supply conditions. The data reinforce the hypothesis that symbiotic association contributes to ionic stability and physiological performance maintenance, consistent with recent findings in vertical farming systems (Zhu & Marcelis, 2023).

A key finding was that intermediate nitrate availability (T3) promoted the highest aerial biomass production (75.3 g per plant), outperforming both T4 and T1. This indicates that moderate nitrogen inputs, especially under symbiotic conditions, are sufficient to maximize productivity while minimizing excess nitrate accumulation. Notably, the nitrate content in edible tissue under T3 was reduced by 29% compared to T4 (1120 vs. 1580 mg kg^{-1}), highlighting the potential to improve food safety through controlled fertilization. These results are consistent with findings from (Demšar et al., 2004; Jokinen et al., 2022; Soufi et al., 2023), which underscore the negative impact of nitrate oversupply on nitrate reductase activity and protein synthesis.

The biomechatronic environment developed in this study showed high functionality by maintaining optimal environmental conditions, including a PPFD of $260 \pm 40 \mu\text{mol m}^{-2} \text{s}^{-1}$, with controlled temperature, humidity, and CO_2 levels, aligned with parameters recommended for efficient *Lactuca sativa* production in controlled agriculture systems (Ahmed et al., 2020). The system's ability to sustain these parameters without frequent manual interventions confirms its technological robustness and ensures that the observed physiological responses were exclusively due to the experimental treatments, reinforcing the scientific validity of the model.

As noted by (Martínez-Moreno et al., 2024), this study reinforces the idea that nutritional efficiency is not solely dependent on nitrate quantity, but rather on its interaction with environmental factors such as light spectrum, interspecies association, and the developmental stage of the plant. Water use efficiency (WUE) was maximized at

T3 under symbiotic conditions, achieving 2.811 g DW L⁻¹, which represents a 38% increase compared to monoculture. This finding underscores the hydraulic and metabolic synergy between nitrogen optimization and biological fixation, resonating with the results reported by (H. Zhang et al., 2022), which indicated gains of 30–40% in WUE within legume-based hydroponic systems.

The results further demonstrate that a moderate nitrogen supply (66% of the standard level), combined with interspecific association with *Medicago sativa*, significantly enhanced photosynthetic and energy efficiency in hydroponic *Lactuca sativa* cultivation. This strategy achieved high quantum efficiency values ($Y(II) = 0.7467$, $F_v/F_m = 0.7591$) and minimal non-photochemical quenching ($NPQ < 0.01$), reflecting a functional and stable PSII. These findings are consistent with those reported by (Mayorga-Gomez et al., 2024), who showed that intermediate nitrogen levels maximize photosynthetic efficiency in hydroponic lettuce, avoiding deficiency or toxicity problems. Similarly, (Ahmed et al., 2020) emphasized the importance of a proper balance of nitrogen, light, and CO₂ to maintain high $Y(II)$ and F_v/F_m indices and minimize NPQ under intensive vertical farming.

Micronutrient dynamics showed inverse trends under excessive nitrate levels. Specifically, zinc (Zn) and manganese (Mn) levels decreased by 33 and 36%, respectively, as nitrate (NO₃⁻) concentrations increased from 66% to 120% (Breš et al., 2022; H. Zhang et al., 2022). This trend likely reflects ionic antagonism and nutrient dilution, highlighting the nutritional trade-offs associated with over-fertilization. Additionally, the lower nickel (Ni) content observed in the fertilized treatments supports the findings of (Jokinen et al., 2022), which noted that micronutrient demand decreases when macronutrient availability is sufficient.

Leaf anatomical traits also responded to nitrate levels. Under deficiency conditions, specific leaf mass (SLM) increased while specific leaf area (SLA) decreased, indicating that stress led to tissue densification. Conversely, SLA improved by 43% when nitrate levels were at 66%, enhancing photosynthetic efficiency. These findings align with previous studies (Kelly et al., 2020; Mickens et al., 2018, 2019; Vaštakaitė-Kairienė & Viršilė, 2015) that connected nitrate status to leaf structure and photoreceptor-mediated responses.

Inoculating *Medicago sativa* with *Rhizobium* increased the foliar nitrogen content in lettuce from 2.4% to 3.2%, demonstrating the effectiveness of plant growth-promoting rhizobacteria (PGPR) in symbiotic relationships (Chen et al., 2018; Y. Wang et al., 2016). This inoculation also led to a 30% reduction in electrical conductivity (EC) in the root zone and improved antioxidant activity, specifically catalase (CAT) and superoxide dismutase (SOD), which increased by 25–40% under stress conditions, highlighting the resilience provided by this plant association (Sabrine et al., 2013).

T6, which involved only lettuce, showed the most concerning combination of low pH (5.3) and high EC (2.20 dS m⁻¹) by the end of the experiment. This pattern indicates that the absence of alfalfa removed essential regulatory mechanisms, such as legumes' ability to regulate pH through alkalizing root exudates (Ramoneda et al.,

2021), as well as their superior efficiency in nutrient uptake (Liu et al., 2021). The observed acidification may be attributed to hydrogen ion exudation by the lettuce roots under ionic stress, while the high EC suggests the accumulation of unconsumed ions, a phenomenon previously reported in hydroponic systems lacking plant diversity (Spencer et al., 2024).

This study also suggests potential avenues for future optimization by modulating the light spectrum, CO₂ concentration, and daily light integral (DLI). Specifically, the use of an 80W20B light recipe has been found to enhance nitrogen content, pigmentation, biomass, and antioxidant accumulation in leafy greens (Mickens et al., 2018, 2019). This improvement is partly attributed to the expression of anthocyanins driven by cryptochromes (Vaštakaitė-Kairienė & Viršilė, 2015).

Collectively, these findings underscore the potential for combining biotechnological tools and ecological strategies in highly controlled environments like vertical farming. This approach creates a new model of sustainable intensification that emphasizes not just yield, but also nutritional efficiency and food safety. This has direct implications for minimizing the risk of nitrate accumulation in fresh produce, as highlighted by (Iammarino et al., 2014; Jokinen et al., 2022).

Finally, based on these findings, future research should combine transcriptomic and metabolomic analyses to identify the molecular pathways associated with nitrogen assimilation efficiency in co-cultivation conditions. Investigating the effects of various LED light spectra (for example, 80% red and 20% blue), along with CO₂ supplementation and DLI values, on the gene expression of key enzymes such as nitrate reductase and glutamine synthetase, will provide a comprehensive understanding of how nutrition, light, plant physiology, and microbial symbiosis interact in vertical farming systems. Extending the evaluation to the postharvest phase—considering aspects like nutraceutical traits, shelf life, and microbial safety—will strengthen the vision of a complete and sustainable value chain for the future of urban agriculture.

VI. CONCLUSIONS

This study provides strong evidence that carefully adjusting nitrate (NO_3^-) concentration in vertical farming NFT (Nutrient Film Technique) systems, combined with agroecological strategies such as intercropping with *Medicago sativa* and inoculating with *Rhizobium*, significantly improves the yield, physiological efficiency, photosynthetic performance, and nutritional quality of *Lactuca sativa* (lettuce). The results revealed that an intermediate nitrate level (designated as T3 or 66% nitrogen strategy) optimized shoot biomass production, enhanced chlorophyll accumulation, improved water use efficiency by 38%, and reduced nitrate accumulation in edible tissues by 29% compared to monoculture systems with nitrogen excess (133%), which notably decreased photosynthetic efficiency by up to 17%.

Moreover, the symbiotic association with *M. sativa* improved rhizospheric conditions by decreasing electrical conductivity and facilitating the uptake of essential micronutrients like zinc (Zn) and manganese (Mn), while simultaneously reducing dependency on synthetic fertilizers. The integration of physiological monitoring of photosystem II (PSII) through real-time automated environmental control further optimized photosynthetic parameters such as $Y(\text{II})$, F_v/F_m , and enhanced light and energy use efficiency (LUE, EUE), while minimizing photoinhibition indicators (NPQ).

These findings highlight the transformative potential of combining biotechnological tools, biomechatronic platforms, and ecological intensification strategies in highly controlled vertical farming environments. This novel approach paves the way toward sustainable intensification models that prioritize not only high yields, but also nutritional efficiency, environmental stewardship, and food safety.

Building on these results, future research should delve into transcriptomic and metabolomic analyses to unravel the molecular pathways underlying nitrogen assimilation, particularly under crop association conditions. It is equally crucial to assess the effects of different LED spectral compositions (e.g., 80% Red and 20% Blue) combined with CO_2 supplementation and varied Daily Light Integral (DLI) values on the expression of key enzymes like nitrate reductase and glutamine synthetase. Parallely, implementing adaptive spectral lighting synchronized with photosynthetic responses, exploring new nitrogen-fixing symbiotic associations, and studying microbiome–plant–sensor interactions in the rhizosphere emerge as promising directions.

Finally, validating the biomechatronic system under commercial-scale conditions, integrating artificial intelligence algorithms for dynamic management based on physiological indicators (such as $Y(\text{II})$ and NPQ), and applying advanced energy-saving technologies (including pulsed lighting and passive cooling) will be fundamental steps to enhance the technical and economic feasibility of vertical farming. Likewise, investigating postharvest phases by considering nutraceutical properties, shelf life, and microbial safety will contribute to building a holistic, resilient, and sustainable vision for the future of urban agriculture.

VI. REFERENCES

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APPENDICE 1



Article

Synergistic Effects of Nitrate on Growth and Ion Balance in Lettuce–Alfalfa Associations under NFT Vertical Farming

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Abstract: Vertical farming systems offer an efficient solution for sustainable food production in urban areas. However, managing nitrate (NO_3^-) levels remains a significant challenge for optimizing crop yield, quality, and safety. This study assessed the effects of five different nitrate concentrations (0, 33, 66, 100, and 133 % of the standard) on the growth, biomass accumulation, and nutrient uptake of *Lactuca sativa* var. *longifolia*, which was cultivated within a multilevel Nutrient Film Technique (NFT) system alongside *Medicago sativa*. The plants were grown in a fully automated vertical farm, equipped with LED lighting ($14.9 \text{ mol m}^{-2} \text{ d}^{-1}$), maintained 1100 ppm CO_2 , and followed a 16-hour photoperiod under controlled conditions. The T3 treatment yielded the highest leaf area (1512.6 cm^2), fresh weight (104.6 g), and water use efficiency (14.4 g L^{-1}), outperforming both the fully fertilized and unfertilized control groups. The co-cultivation with **Medicago sativa** helped stabilize root-zone pH (ranging from 6.1 to 6.5) and reduced electrical conductivity (EC) to 2.04 dS m^{-1} , indicating enhanced nutrient regulation through biological nitrogen fixation. Additionally, foliar nitrate content decreased from 1580 to 1120 mg kg^{-1} without any reduction in yield, and micronutrient up-take (specifically Zn and Mn) improved at moderate NO_3^- levels. The leaf structure also benefited from balanced nitrogen, promoting better photosynthetic performance. Future re-search should focus on enhancing the automation systems used in this study, particularly by incorporating real-time monitoring and adjustments to nutrient dosing. This would facilitate more precise control of environmental factors like pH and EC, ultimately helping to develop crop-specific protocols for intercropping in hydroponic vertical farms.

Keywords: Vertical Farming (VF); Nutrient Film Technique (NFT); *Lactuca sativa*; *Medicago sativa*; Nitrate availability; Ions; Macronutrients; Micronutrients.

1. Introduction

Vertical farms (VF) represent an innovative solution that transforms traditional food production by efficiently adapting to densely populated urban environments [1]. These

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agricultural systems utilize multi-layered structures in controlled environments and incorporate advanced technologies such as hydroponics, LED lighting, and automated environmental control systems. This integration allows for optimized space usage, increased crop yields, and enhanced resource efficiency, enabling the continuous production of high-value, perishable crops like leafy vegetables and herbs, especially in urban settings [2]. However, the success of vertical farming depends not only on its engineering design but also on its ability to improve key physiological processes in plants under controlled conditions. Factors such as nutrient management—particularly nitrogen dynamics—light quality and intensity, temperature, and water availability can be carefully regulated in these systems [3]. This opens up new opportunities to enhance photosynthetic efficiency, reduce abiotic stress, and optimize nutrient uptake in species such as *Lactuca sativa* (lettuce).

Nitrogen is an essential element for the biosynthesis of amino acids, nucleic acids, and chlorophyll, making it a primary limiting factor in plant growth. However, improper nitrogen management—especially the commonly used form of nitrate (NO_3^-)—can lead to significant physiological and biochemical changes in both conventional and advanced hydroponic systems [4–6]. In vertical farms, the availability of nitrogen and the intensity of light directly affect photosynthetic efficiency and the accumulation of nitrates in edible plant tissues. Research has shown that, under low LED light intensity, leaves tend to accumulate nitrates when the reduction rate is insufficient for converting them into proteins [7,8].

In Nutrient Film Technique (NFT) systems, where the nutrient solution circulates continuously, nitrate (NO_3^-) concentrations can significantly impact ionic absorption, root development, and nutrient translocation. In this study, the nitrate levels were not actively controlled during circulation, which may pose a limitation when interpreting the specific physiological responses related to nitrogen availability [9].

Previous research has demonstrated that altering nitrate (NO_3^-) concentrations in nutrient solutions can significantly impact biomass production, photosynthetic efficiency, nitrate reductase activity, and the uptake of essential ions such as Ca^{2+} , K^+ , and Mg^{2+} . These factors are all crucial for the organoleptic and nutritional quality of leafy vegetables like *Lactuca sativa* [10–13]. However, most studies have focused on cultivation systems other than the Nutrient Film Technique (NFT), typically examining static or batch hydroponic conditions. This indicates a need to verify whether similar physiological responses occur in continuous-flow systems like NFT. In modified NFT setups, it was found that suspending nitrogen fertilization 2 to 4 days before harvest could reduce foliar nitrate accumulation by 29 to 58% without affecting yield [14]. Additionally, dynamic NO_3^- dosing strategies implemented in aeroponic systems have achieved reductions of up to 92% in leaf nitrate based on varying light conditions [7].

Although previous research has primarily focused on timing-based adjustments, there has been limited investigation into the effects of fixed nitrate concentration gradients, particularly in continuous-flow nutrient film technique (NFT) systems. This study aims to address this knowledge gap by evaluating the physiological and nutritional responses of *Lactuca sativa* (lettuce) to stable nitrate (NO_3^-) levels in a closed NFT system, which incorporates the symbiotic interaction with *Medicago sativa* (alfalfa). This intercropping system, which combines alfalfa and lettuce, presents a strategic agronomic solution. It leverages alfalfa's ability to fix nitrogen in the soil and enhance soil structure [15–17], while also benefiting from the rapid growth and commercial value of lettuce.

Research shows that this intercropping method, especially in soil-based systems, improves nitrogen use efficiency, increases total biomass yield, and decreases dependency on synthetic nitrogen inputs. It achieves this through biological nitrogen fixation and complementary nutrient uptake [18,19].

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Intercropping *Medicago sativa* and *Hordeum marinum* resulted in increased biomass accumulation and improved nutrient uptake. Additionally, this method led to a reduction in soil pH and electrical conductivity under salinity conditions of both 0 and 150 mM NaCl [20]. In soilless systems, the Nutrient Film Technique (NFT) has proven to be highly effective in managing nutrient solutions. It allows for precise dosing of nitrates and effective control of variables such as pH and electrical conductivity [21,22]. These benefits position NFT as a crucial technology for short-cycle crops with high nutrient demands, such as lettuce.

A complementary agroecological strategy involves the co-inoculation of legumes with *Rhizobium* and plant growth-promoting bacteria (PGPR). This approach enhances biological nitrogen fixation (BNF), increases nutrient availability, and improves the health of the rhizosphere [23–25]. This synergistic effect is especially significant in controlled environments, where factors such as artificial light, oxygenation, and substrate type can influence microbial activity and nodulation.

In greenhouse conditions, the intercropping of *Lactuca sativa* (lettuce) with *Medicago sativa* (alfalfa) has been studied for its potential to enhance biomass accumulation, improve nutrient use efficiency, and increase postharvest quality. A randomized field experiment conducted under controlled conditions demonstrated that incorporating alfalfa into the soil, along with solid nitrogen fertilization at rates of 200–300 kg per hectare, significantly improved yield [26]. This approach also resulted in a reduction of nitrate (NO₃⁻) content in the sap and optimized the uptake of potassium and calcium in lettuce.

Recent studies indicate that the concurrent cultivation of *Medicago sativa* (alfalfa) and *Lactuca sativa* (lettuce) under conditions of heavy metal stress, specifically lead (Pb) and cadmium (Cd), significantly enhances the antioxidant response in lettuce [27]. Furthermore, findings demonstrate that *Medicago sativa* modifies the rhizosphere microenvironment of lettuce within hydroponic greenhouse systems. This modification not only facilitates lettuce's tolerance to nanoparticles but also enhances its overall metabolic performance [28].

Soilless cultivation of *L. sativa*, or lettuce, presents certain phytosanitary challenges. Pathogens such as *Pythium* spp., *Fusarium* spp., *Erwinia*, and *Pseudomonas* thrive in controlled environments. A study reported an increased risk of human pathogens, like *Salmonella*, being absorbed into hydroponic lettuce if factors such as pH, aeration, and water quality are not properly managed [5]. To help reduce this risk, incorporating legumes like *M. sativa* has been suggested, as they can promote microbial diversity and positively influence the rhizosphere.

Vertical Farms (VF) exemplify the highest level of technological integration in agriculture. By combining LED lighting, automated CO₂ control, and precise nutrient management, these farms enable unparalleled accuracy in studying interspecific interactions. Singh et al. [29] reported that supplementing CO₂ in Nutrient Film Technique (NFT) vertical farms increased both the yield and mineral content of lettuce. This finding confirms that such an environment serves as an ideal model for evaluating complex interactions, like the alfalfa-lettuce association, under controlled conditions.

Investigations have documented the effects of nitrogen on foliar morphophysiological parameters. Adequate nitrogen availability has been shown to increase specific leaf area (SLA) and enhance photosynthetic efficiency [30,31]. It also raises intracellular osmotic pressure, which promotes cell expansion and overall vegetative development. Furthermore, water use efficiency (WUE) is associated with nitrogen concentration, indicating that a balanced supply of nitrogen optimizes both water usage and metabolic processes in high-yield plants [32,33].

Even though there have been advancements in nutrient management for hydroponic systems, there are still gaps in the literature regarding the effects of different nitrate

concentrations on the physiology of *Lactuca sativa*, particularly in nutrient film technique (NFT) systems utilized in vertical farms. Limited studies have examined ionic accumulation, nutrient efficiency, and the morphophysiological responses of lettuce when intercropped with legumes such as *Medicago sativa*. Furthermore, most research has concentrated on monoculture systems, overlooking how intercropping influences nutrient availability and absorption. Additionally, there is a lack of documentation on how these interactions respond under controlled conditions with specific variations in nitrate concentration, especially concerning growth, biomass production, and nutritional quality.

This study aims to evaluate the effects of different nitrate (NO_3^-) concentrations in nutrient solutions on the growth, biomass production, and ion accumulation in *Lactuca sativa* (LS) var. *longifolia* when cultivated in a Nutrient Film Technique (NFT) system alongside *Medicago sativa* (MS). A carefully controlled experimental design was employed to assess the specific impact of each nitrate concentration on various aspects of plant development.

2. Results

This study evaluated how nitrate availability influences growth and nutrient uptake in vertical NFT systems using lettuce–alfalfa associations. Results on biomass, ion dynamics, and nutrient efficiency under varying nitrate levels are presented below.

2.1. Nutritive Solution Dynamics

2.1.1. Water Consumption

Figure 1 illustrates the water consumption patterns across treatments during the four-week cultivation period. Treatments T1 and T2 demonstrated similar total water use, averaging 39.4 L each. In contrast, T3, T4, and T5 showed slightly higher water retention, averaging 41.3 L. Among all treatments, T1 exhibited the highest overall consumption, reducing its water volume to 23.7 L, while T4 and T5 retained the most water, both ending at 27.4 L. By the end of the experiment, water levels in T1 and T2 were the lowest, with 11.1 L and 10.2 L, respectively, compared to the control (T0) which remained at 12.1 L. Notably, T2 experienced the sharpest decline in water availability, while T5 retained the highest final volume, indicating a potential dose-dependent effect related to treatment application.

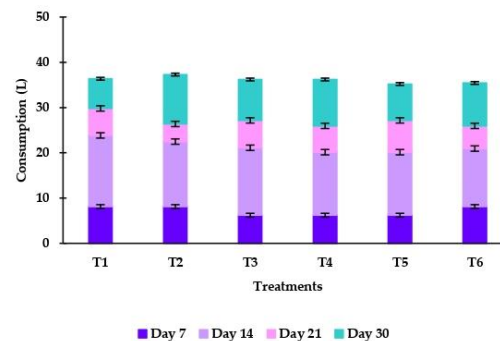


Figure 1. Weekly water consumption per treatment throughout the four-week experimental period.

2.1.2. pH dynamics.

The pH dynamics across the treatments, as shown in Table 1, exhibited fluctuations over the four-week period, with adjustments made to maintain optimal ranges (5.5–6.5 for hydroponic low salinity and medium salinity). The T6 group consistently acidified the nutrient solution by the second, third, and fourth weeks. In contrast, sharp increases in pH were observed in treatments T1 (6.87), T2 (7.36), T3 (6.92), and T5 (7.15). Treatment T4 experienced a smaller increase in pH, reaching 6.25. The alfalfa treatments stabilized between 6.0 and 6.5, except for T2, which remained slightly elevated at 6.46. Notably, treatment T4 required minimal adjustments.

Table 1. Variation in nutrient solution pH across treatments over the course of four weeks (W).

Treatments	W0 (Day 0)	W1		W2		W3		W4(Day 30)
		Initial	Final	Initial	Final	Initial	Final	
T1	6.02 ^a	6.87 ^c	5.99 ^a	6.88 ^c	6.02 ^a	6.21 ^c	6.16 ^a	6.06 ^b
T2	6 ^a	7.36 ^a	5.94 ^a	7.33 ^a	6.06 ^a	6.85 ^a	6.06 ^b	6.46 ^a
T3	6.01 ^a	6.92 ^c	5.98 ^a	6.99 ^b	6.03 ^a	6.85 ^a	6.01 ^b	6.13 ^b
T4	5.99 ^a	6.25 ^d	6.04 ^a	6.24 ^d	6.09 ^a	6.25 ^c	6.06 ^b	6.09 ^b
T5	6.01 ^a	7.15 ^b	6 ^a	6.9 ^c	6.04 ^a	6.59 ^b	6.03 ^b	6.13 ^b
T6	6 ^a	6.04 ^c	6.04 ^a	5.37 ^e	5.9 ^a	5.5 ^d	5.9 ^b	5.3 ^c

2.1.2. Electrical Conductivity (EC)

Figure 2 shows that the electrical conductivity (EC) values gradually increased across all treatments, indicating the accumulation of ions as water was consumed. By the fourth week of the experiment, the control group exhibited the highest EC at 2.20 dS m⁻¹. In comparison, Treatments T1 and T2 recorded lower final EC values of 2.04 dS m⁻¹, which aligned with their higher water consumption (as mentioned in Section 1.1). Treatments T4 and T5 reached an EC of 2.06 dS m⁻¹, suggesting effective nutrient uptake. Despite these increasing trends, the EC levels remained within the expected range throughout the experiment, eliminating the need for adjustments and ensuring stable nutrient availability.

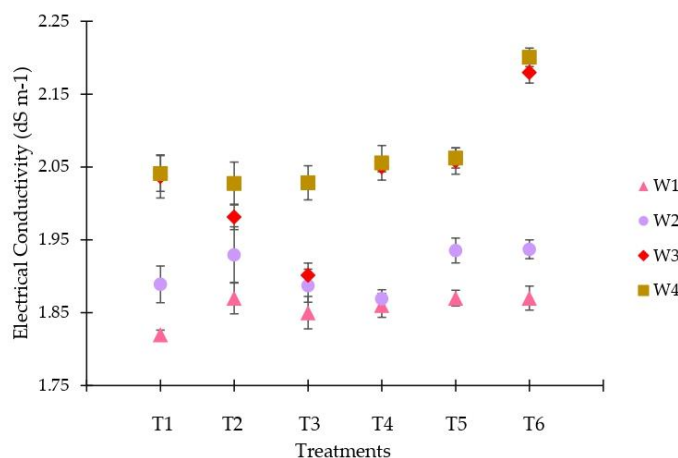


Figure 2. EC of the nutrient solution by treatment over the four-week experimental period.

2.2. Plant Growth and Biomass Accumulation.

Table 2 presents the data on fresh and dry biomass accumulation across treatments. An increase in both fresh and dry mass was observed with higher levels of nitrogen fertilization. The total fresh weight of the plants increased notably, rising from 37.21 g in treatment T1 to 136.75 g in treatment T5, with the control group exhibiting the highest weight at 147.83 g. Notably, the leaf fresh weight in treatments with higher nitrogen levels reached up to 92.9% in T5 compared to the control, whereas T1 had the lowest fresh weight percentage (77.9%) and a comparatively higher dry weight percentage (22.1%). This pattern suggests that increased nitrogen availability promotes greater water accumulation in plant tissues. Additionally, the mass lost during drying followed the same trend, being significantly lower in T1 (12.84 g) and highest in the control group (104.71 g), further indicating enhanced tissue hydration due to nitrogen fertilization.

Table 2. Effect of treatments on leaf area, specific leaf mass (SLM), leaf number, fresh mass, dry mass, root biomass, and water use efficiency (WUE) in lettuce plants at 30 days after transplanting.

Plant Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
						
Leaf number	16.50 ^b	29.00 ^a	30.33 ^a	29.00 ^a	24.83 ^b	28.67 ^{ab}
Leaf area (cm ²)	344.90 ^c	1297.14 ^{ab}	1512.62 ^a	1041.95 ^b	1173.23 ^b	1357.75 ^{ab}
Fresh mass (g)	17.93 ^c	88.30 ^{ab}	104.57 ^{ab}	83.55 ^b	98.79 ^{ab}	114.60 ^a
Dry mass (g)	5.09 ^b	7.63 ^{ab}	8.95 ^a	7.63 ^{ab}	7.55 ^{ab}	9.90 ^a
Root mass (g)	19.27 ^c	28.58 ^b	33.93 ^{ab}	34.90 ^{ab}	37.97 ^a	33.23 ^{ab}
SLM (mg cm ⁻²)	15.23 ^a	6.02 ^b	5.91 ^b	7.363 ^b	6.48 ^b	7.33 ^b
WUE (gDW L ⁻¹)	1.572 ^b	2.242 ^{ab}	2.811 ^a	2.395 ^{ab}	2.522 ^{ab}	3.264 ^a

Figure 3 depicts the changes in root mass and the proportion of root biomass across different treatments. The root mass showed a consistent upward trend, increasing from 19.27 g in treatment T1 to 37.97 g in T5, with T6 recording a root mass of 33.23 g. This trend indicates an enhanced ability for water and nutrient uptake when nitrate (NO_3^-) availability is higher. However, the percentage of root biomass (% root) relative to the total plant mass decreased as NO_3^- levels increased—falling from 52 % in T1 to 28 % in T5 and reaching a low of 22 % in T6. This decline suggests that, under low nitrogen availability conditions, plants invest a greater proportion of their biomass in root development to maximize nutrient acquisition. In contrast, higher nitrate levels promote a shift in biomass allocation toward the shoots, which increases the proportion of aboveground fresh weight (% FW).

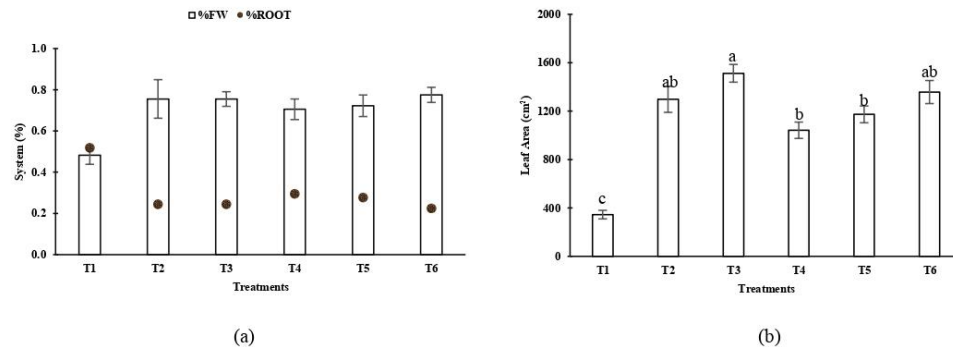


Figure 3. Effects of nitrate availability on biomass allocation and leaf development in lettuce. (a) Proportion of fresh weight (%FW) versus root weight (%Root) across treatments. (b) Leaf area variation per treatment at 30 days after transplanting.

In treatment T1, plants displayed the lowest number of leaves, averaging 16.50, while treatment T3 had the highest count at 30.33 leaves. Treatment T6 followed closely with 28.67 leaves, and T2 had 29.00 leaves. The total leaf area significantly increased with the application of nitrate. T1 had a leaf area of 344.90 cm², whereas the nitrate treatments ranged from 1,041.95 cm² in T4 to 1,512.62 cm² in T3. The control group reached a leaf area of 1,357.75 cm². Leaves from T1 exhibited a significantly higher specific leaf mass (SLM) of 14.75 mg/cm², indicating that they were denser and thicker, likely due to nitrate deficiency. In contrast, the nitrogen fertilization treatments showed an SLM ranging from 5.88 to 7.32 mg/cm², which suggests that these leaves were thinner and broader, optimizing light capture. WUE was significantly lower in T1, at 1.572 g dry weight (DW) per liter of water (H₂O), compared to the fertilized treatments. T6 attained the highest efficiency at 3.264 g DW per liter of H₂O, followed by T3 with 2.811 g DW per liter of H₂O.

2.3. Accumulation of Mineral Elements in Plant Tissue.

Table 3 summarizes the macronutrient content across treatments. A significant difference was observed in treatment T1, where levels of calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), sulfur (S), and nitrogen (N) were all notably lower per unit of dry biomass compared to other treatments. In contrast, the remaining treatments displayed macronutrient concentrations comparable to the control group. Nitrogen (N) content exhibited clear statistical differences. The lowest value was recorded in T1 (12.8c),

while fertilized treatments reached significantly higher values, peaking in T4 (43.8a).
Treatments T5 (41.3ab) and T6 (40.4ab) did not differ significantly from each other, sug-
gesting that nitrogen uptake may plateau beyond a certain threshold of availability. Simi-
larly, phosphorus (P) content varied significantly across treatments. T1 had the lowest
value (3.8d), while the highest concentrations were found in T5 (11.3a) and T6 (11.2a).
Intermediate values were observed in T2 (10.1b) and T3 (8.6c), indicating that P uptake
improves with nitrogen availability, up to a saturation point.

Table 3. Macronutrient and micronutrient accumulation in lettuce plants harvested 30 days after
transplanting. Values represent means per treatment. Different letters indicate statistically signifi-
cant differences ($p \leq 0.05$) according to Tukey's multiple comparison test.

NUTRIENTS (mg g ⁻¹ DW)		Treatments						Literature Ranges		
		T1	T2	T3	T4	T5	T6	min	max	References
Macronutrients	Nitrogen (N)	12.8 ^c	40.4 ^{ab}	38.6 ^b	43.8 ^a	41.3 ^{ab}	40.4 ^{ab}	24	40	[12,34,35]
	Phosphorus (P)	3.8 ^d	10.1 ^b	8.6 ^c	10.9 ^{ab}	11.3 ^a	11.2 ^a	6	9.5	[36–38]
	Potassium (K)	37.3 ^c	85.2 ^a	77.2 ^b	79 ^{ab}	82.6 ^{ab}	77.6 ^b	55	85	[36,39]
	Calcium (Ca)	6.3 ^c	13.2 ^a	11.7 ^b	11.1 ^b	11.3 ^b	11.8 ^b	9	14	[40,41]
	Magnesium (Mg)	2 ^c	3.8 ^a	3.3 ^b	3.7 ^a	3.7 ^a	3.1 ^b	2.5	4	[42,43]
	Sulfur (S)	1.5 ^d	2.6 ^{bc}	2.4 ^c	3.0 ^a	2.9 ^a	2.8 ^{ab}	1.8	2.6	[34,44]
Micronutrients	Iron (Fe)	19.5 ^d	39.1 ^b	34.8 ^c	39.2 ^b	42 ^b	48.3 ^a	24	35	[42,45,46]
	Zinc (Zn)	65.3 ^c	241 ^{ab}	262 ^a	244 ^{ab}	223 ^b	234 ^b	120	190	[47,48]
	Manganese (Mn)	239 ^d	374 ^c	477 ^a	419 ^b	373 ^c	367 ^c	310	450	[34,46]
	Copper (Cu)	3.16 ^d	9.97 ^c	9.63 ^c	11.5 ^b	11.2 ^b	12.9 ^a	6	10	[34,46]
	Boron (B)	30.6 ^d	42.5 ^a	36.4 ^b	42.3 ^a	34.8 ^{bc}	31.5 ^{cd}	28	40	[46,49]
	Nickel (Ni)	5.52 ^a	0.37 ^c	0.16 ^c	0.22 ^c	0.2 ^c	0.64 ^b	0.15	0.35	[47,50,51]
Molybdenum (Mo)		0.1 ^b	0.12 ^a	0.06 ^d	0.05 ^e	0.09 ^c	0.1 ^b	0.04	0.09	[46,52]

Potassium (K) showed a significant response to fertilization, increasing from 37.3 mg
kg⁻¹ at T1 to a maximum of 85.2 mg kg⁻¹ at T2. However, the higher treatments (T3, T4, and
T5) displayed lower potassium levels, measuring 77.2 mg kg⁻¹, 79.0 mg kg⁻¹, and 82.6 mg
kg⁻¹, respectively. Treatment T6 also had a similar value of 77.6 mg kg⁻¹. These results in-
dicate that potassium uptake is more efficient at moderate levels of nitrogen fertilization,
and no significant increases in potassium are observed at higher dosages. Calcium (Ca)
recorded its lowest value at T1, with a concentration of 6.3 mg kg⁻¹, and reached its peak
at T2 with 13.2 mg kg⁻¹. The subsequent treatments with fertilization showed similar cal-
cium values ranging from 11.1 mg kg⁻¹ to 11.8 mg kg⁻¹, with no significant differences be-
tween them. This suggests that calcium uptake stabilizes after reaching a certain level of
available nitrogen.

Magnesium (Mg) demonstrated a similar trend, with the lowest value recorded at T1
(2c) and the highest values observed at T2 and T4 (3.8a and 3.7a, respectively). The values
in the other treatments were intermediate, measuring 3.3b at T3 and 3.1b at T6. This pat-
tern suggests that nitrogen availability enhances magnesium uptake to some extent.

In terms of sulfur (S), the lowest value was also noted at T1 (1.5d), which increased
with fertilization, reaching its peak at T4 (3a). Although the values at T5 and in the control
treatment were somewhat lower (2.9a and 2.8ab, respectively), there was no significant
difference from T4. This indicates that sulfur responds positively to nitrogen up to a cer-
tain level.

Micronutrient levels varied with different nitrogen fertilization treatments, showing significant statistical differences among them. Iron (Fe) concentrations were lowest in the T1 treatment, at 19.5d, but increased with nitrogen fertilization, reaching the highest level in the control group at 48.3a. The T2, T4, and T5 treatments displayed intermediate iron values of 39.1b, 39.2b, and 42b, respectively. There were no significant differences among these three treatments, suggesting that while nitrogen availability enhances iron uptake, other factors may limit its absorption beyond a certain point.

Zinc (Zn) exhibited its lowest concentration at T1 (65.3c) and reached its peak at T4 (262a). The zinc levels for T2 and T4 were similar, recorded at 241ab and 244ab, respectively. In contrast, T5 and the control group showed lower levels at 223b and 234b. This indicates that zinc uptake is higher at intermediate fertilization levels but tends to decrease at elevated levels.

Manganese (Mn) had its lowest concentration at T1 (239d) and peaked at T3 (477a). T4 displayed an intermediate value of 419b, while treatments T2, T5, and T6 fell within a similar range of 373c to 374c. This suggests that nitrogen availability enhances manganese uptake up to T3, but beyond that, the uptake declines.

Copper (Cu) exhibited a pattern similar to that of other nutrients, showing its lowest value at T1 (3.16d) and peaking at T6 (12.9a). Treatments T4 and T5 displayed intermediate values of 11.5b and 11.2b, respectively, while T2 and T3 had significantly lower values at 9.97c and 9.63c. These results suggest that copper uptake improves with fertilization; however, the control group exhibited even higher uptake, potentially due to other environmental factors.

Boron (B) reached its highest level at T2 (42.5a), with similarly high values observed at T4 (42.3a). In contrast, T5 showed a significant decrease (34.8bc), and T1 recorded the lowest value (30.6d). This indicates that boron uptake is optimal at moderate levels of nitrogen fertilization but tends to decline when nitrogen is in excess.

Nickel (Ni) exhibited a pattern that contrasts with most other nutrients. It reached its highest value at T1 (5.52a) but experienced a significant decline in the fertilization treatments, dropping to its lowest level at T3 (0.16c). Although T6 recorded a higher value (0.64b), it was still significantly lower than T1. This suggests that the availability of nitrogen decreases nickel uptake, likely because the presence of sufficient available nitrogen reduces the need for this element.

In contrast, molybdenum (Mo) showed its highest uptake at T2 (0.12a), while T4 registered the lowest value (0.05e). The other treatments displayed intermediate values, with T6 at 0.1b and T5 at 0.09c. This indicates that molybdenum uptake is negatively impacted by high levels of nitrogen fertilization, possibly due to competition with other anions in the nutrient solution.

2.4. Energy Consumption

Table 4 presents the monthly energy consumption of the main subsystems in the vertical farming setup. Among all components, the temperature control systems were the most energy-intensive, consuming 618.16 kWh month⁻¹, thus representing the largest contributor to total energy demand. The lighting systems followed with a consumption of 293.76 kWh month⁻¹, underscoring their substantial energy requirements despite the use of high-efficiency LED fixtures. The irrigation subsystem, which includes pumps and nutrient dosing systems, consumed 99.72 kWh month⁻¹, while CO₂ injection accounted for a comparatively minor 8.20 kWh month⁻¹.

Finally, the control and monitoring system consumed 33.80 kWh month⁻¹, reflecting the baseline energy needed for automation and data collection within the production environment.

Table 4. Monthly Energy Consumption by Subsystems in the Vertical Farming System.

Subsystem	Energy Consumption (kWh month ⁻¹)	Percentage of Total (%)
Temperature	618.16	58.7
CO ₂	8.20	0.8
Lights	293.76	27.9
Irrigation	99.72	9.5
System	33.80	3.2

3. Discussion

The results clearly indicate that the availability of nitrate (NO₃⁻) in vertical hydroponic nutrient film technique (NFT) systems significantly influences the physiological, nutritional, and morphological development of *Lactuca sativa* (lettuce) when grown alongside *Medicago sativa* (alfalfa). These findings provide quantifiable evidence to address a complex issue: determining the optimal nitrate levels in controlled crop environments while balancing productivity and the nutritional quality of the final product. The presence of *Medicago sativa* not only modulated physicochemical parameters—such as pH (6.1–6.7) and electrical conductivity (EC, which decreased from 2.1 to 1.5 mS cm⁻¹)—but also enhanced nutrient absorption and the balance within the rhizosphere. This modulation likely improved ionic availability and nutrient uptake efficiency, as supported by references [53,54].

A key finding was that intermediate nitrate availability (T3) promoted the highest aerial biomass production (75.3 g per plant), outperforming both T4 and T1. This indicates that moderate nitrogen inputs, especially under symbiotic conditions, are sufficient to maximize productivity while minimizing excess nitrate accumulation.

Notably, the nitrate content in edible tissue under T3 was reduced by 29% compared to T4 (1120 vs. 1580 mg kg⁻¹), highlighting the potential to improve food safety through controlled fertilization. These results are consistent with findings from [7,13,55], which underscore the negative impact of nitrate oversupply on nitrate reductase activity and protein synthesis. As noted by Martínez-Moreno et al. [12], this study reinforces the idea that nutritional efficiency is not solely dependent on nitrate quantity, but rather on its interaction with environmental factors such as light spectrum, interspecies association, and the developmental stage of the plant.

Water use efficiency (WUE) was maximized at T3 under symbiotic conditions, achieving 2.811 g DW L⁻¹, which represents a 38% increase compared to monoculture. This finding underscores the hydraulic and metabolic synergy between nitrogen optimization and biological fixation. It resonates with the results reported by Zhang et al. [56], which indicated gains of 30–40% in WUE within legume-based hydroponic systems.

Micronutrient dynamics showed inverse trends under excessive nitrate levels. Specifically, zinc (Zn) and manganese (Mn) levels decreased by 33 and 36%, respectively, as nitrate (NO₃⁻) concentrations increased from 66% to 120% [10,56]. This trend likely reflects ionic antagonism and nutrient dilution. These findings highlight the nutritional trade-offs associated with over-fertilization, which can impact both plant health and consumer quality. Additionally, the lower nickel (Ni) content observed in the fertilized treatments supports the findings of Jokinen et al. [55], which noted that micronutrient demand decreases when macronutrient availability is sufficient.

Leaf anatomical traits also showed a response to nitrate levels. Under deficiency conditions, specific leaf mass (SLM) increased while specific leaf area (SLA) decreased, indicating that stress led to tissue densification. Conversely, SLA improved by 43% when

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nitrate levels were at 66%, which enhanced photosynthetic efficiency. These findings align with previous studies with [30–32] that connected nitrate status to leaf structure and photoreceptor-mediated responses.

Inoculating *Medicago sativa* with *Rhizobium* increased the foliar nitrogen content in lettuce from 2.4% to 3.2%, demonstrating the effectiveness of plant growth-promoting rhizobacteria (PGPR) in symbiotic relationships [57,58]. This inoculation also led to a 30% reduction in electrical conductivity (EC) in the root zone and improved antioxidant activity, specifically catalase (CAT) and superoxide dismutase (SOD), which increased by 25–40% under stress conditions. These findings highlight the resilience provided by this plant association [59].

This study demonstrates that growing *Lactuca sativa* (lettuce) alongside *Medicago sativa* (alfalfa) in vertical NFT (nutrient film technique) systems enhances resource use efficiency, such as water and nutrients, while also improving the nutritional quality and sustainability of the final product. The nitrate concentration in the edible tissue was found to be 1580 mg kg⁻¹ in the 100% NO₃⁻ treatments, but it was reduced to 1120 mg kg⁻¹ in the T3 treatment, while maintaining similar yields. This 29 % reduction in nitrate content represents a significant advancement in food safety.

Furthermore, the study highlights the important role of legumes in improving root zone conditions, showcasing the potential for innovative and sustainable agricultural practices to tackle the challenges of modern food production. This research also addresses concerns raised by previous studies regarding the health risks associated with nitrate overaccumulation [55,60]. Therefore, this study not only enhances production efficiency but also improves consumer safety in vertical hydroponic agriculture.

T6, which involved only lettuce, showed the most concerning combination of low pH (5.3) and high electrical conductivity (EC) (2.20 dS m⁻¹) by the end of the experiment (see Tables 2 and 3). This pattern indicates that the absence of alfalfa removed essential regulatory mechanisms, such as legumes' ability to regulate pH through alkalizing root exudates [61], as well as their superior efficiency in nutrient uptake [54]. The observed acidification may be attributed to hydrogen ion exudation by the lettuce roots under ionic stress, while the high EC suggests the accumulation of unconsumed ions (such as potassium and sulfate), a phenomenon previously reported in hydroponic systems lacking plant diversity [62]. Future studies should analyze the ionic composition of the solution to validate this hypothesis.

The study suggests potential avenues for future optimization by modulating the light spectrum, CO₂ concentration, and daily light integral (DLI). Specifically, the use of an 80W20B light recipe has been found to enhance nitrogen content, pigmentation, biomass, and antioxidant accumulation in leafy greens [30,63]. This improvement is partly attributed to the expression of anthocyanins driven by cryptochromes [31].

This study provides strong evidence that precisely modulating nitrate (NO₃⁻) concentrations in vertical Nutrient Film Technique (NFT) systems, combined with agroecological practices such as intercropping with *Medicago sativa* and inoculation with *Rhizobium*, significantly enhances the yield, physiological efficiency, and nutritional quality of *Lactuca sativa*. An intermediate level of nitrate availability (T3) was found to be optimal for shoot biomass production, resulting in a 29% reduction in nitrate accumulation in edible tissues and a 38% increase in water use efficiency compared to monoculture systems. Additionally, the symbiosis with *M. sativa* improved conditions in the rhizosphere, reduced electrical conductivity, and facilitated the uptake of essential micronutrients like zinc (Zn) and manganese (Mn), thereby mitigating the negative effects of excessive NO₃⁻.

The simultaneous inoculation with plant growth-promoting rhizobacteria (PGPR) also increased foliar nitrogen content, antioxidant levels, and markers of metabolic resilience, contributing to a more robust system capable of withstanding abiotic stress.

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Collectively, these findings underscore the potential for combining biotechnological tools and ecological strategies in highly controlled environments like vertical farming. This approach creates a new model of sustainable intensification that emphasizes not just yield, but also nutritional efficiency and food safety. This has direct implications for minimizing the risk of nitrate accumulation in fresh produce, as highlighted by sources [55,64] Based on these findings, future research should combine transcriptomic and metabolomic analyses to identify the molecular pathways associated with nitrogen assimilation efficiency in co-cultivation conditions. Additionally, it is recommended to investigate the effects of various LED light spectra (for example, 80% red and 20% blue), along with CO₂ supplementation and Daily Light Integral (DLI) values, on the gene expression of key enzymes such as nitrate reductase and glutamine synthetase. These research directions will provide a comprehensive understanding of how nutrition, light, plant physiology, and microbial symbiosis interact in vertical farming systems. Finally, extending the evaluation to the postharvest phase—considering aspects like nutraceutical traits, shelf life, and microbial safety—will strengthen the vision of a complete and sustainable value chain for the future of urban agriculture.

4. Materials and Methods

Figure 4 illustrates the experimental design used to evaluate the effects of different nitrogen concentrations on the growth of *Lactuca sativa* var. *longifolia* in a hydroponic Nutrient Film Technique (NFT) system, in co-culture with *Medicago sativa* (alfalfa). This experiment was conducted under artificial lighting and automated environmental control. *Medicago sativa* was sown and treated with nitrogen-fixing bacteria at three specific stages: pre-planting (10⁶ CFU/mL), the second week post-transplanting (10⁸ CFU/mL), and the fourth week (10⁶ CFU/mL). *Lactuca sativa* seedlings, commercially acquired at three weeks of age, were acclimatized in a nursery system for one week prior to co-transplantation. Both species were then transplanted into NFT channels, under controlled LED lighting conditions (270 ± 30 µmol m⁻² s⁻¹), and were exposed to nutrient solutions with varying nitrogen concentrations. Six treatments (T1–T6) were established, representing nitrogen levels of 0%, 33%, 66%, 100%, and 133%. Treatments T1 to T5 were implemented as co-cultures of lettuce and alfalfa (5 plants of each species per treatment), while T6 served as the control, consisting of a monoculture of lettuce (10 plants) under 100% nitrogen. Throughout the experiment, pH, electrical conductivity (EC), and water consumption (WC) of the nutrient solution were monitored daily. At the end of the cycle, the response variables for *Lactuca sativa* included fresh weight, leaf area (measured via digital scanning), dry weight (measured after dehydration), and tissue concentrations of macro- and micronutrients. This experimental design allowed for the assessment of the nutritional and physiological efficiency of romaine lettuce when cultivated in association with alfalfa and subjected to varying nitrogen regimes. The study incorporated automated environmental controls and precise methods for quantifying growth.

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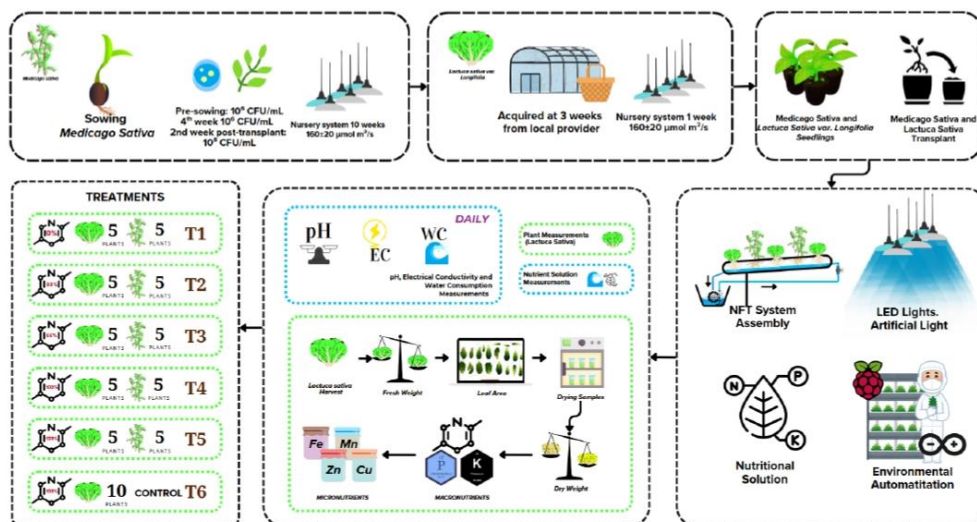


Figure 4. General scheme of the experiment for growing.

4.1. Plant Material

Three-week-old seedlings of lettuce (*Lactuca sativa* L. *Longifolia*, LS) were sourced from a local provider in Asientos, Aguascalientes, Mexico. These plants were kept in a nursery system for one week under light conditions of $160 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$ before being transplanted into the hydroponic system. Certified seeds of *Medicago sativa* (MS) variety San Miguelito, which had germination and purity rates of 90% and 98%, respectively, were sown in a 60-cavity black plastic seedbed measuring $20 \times 60 \times 5$ cm. The MS plants were transplanted into the same hydroponic system at the tenth week, achieving a height of 25 ± 5 cm after their first cut.

Before sowing, the MS seeds were immersed in a suspension of *Rhizobium* at a concentration of 10^6 colony-forming units CFU mL^{-1} . The MS seedlings were also exposed to *Rhizobium* again in the second and fourth weeks after germination at the same concentration. Finally, in the second week after transplanting, *Rhizobium* was supplied at a concentration of 10^8 CFU mL^{-1} to ensure adequate colonization and symbiosis with the plants.

4.2. Biofertilizers Used in the Experiment.

Five experimental conditions were established based on nutrient solution balances to modify nitrate levels (see Table 1). Municipal tap water from TecNM | Campus Pabellón de Arteaga was analyzed to determine its anionic and cationic composition. This analysis was conducted by Fertilab | Agricultural Laboratory, located in Cd. Industrial de Celaya, Celaya, Gto, Mx. Based on these results, a suitable nutrient solution balance for both plant species *Lettuca Sativa* (LS) and *Medicago sativa* (MS) was formulated. All solutions were balanced to maintain an electrical conductivity (EC) of $2.0 \pm 0.5 \text{ mS cm}^{-1}$ and a pH of 6.0 ± 0.5 .

Table 4. Distribution of treatments and relative percentage of NO_3^- used

Treatments	NO_3^- (%)	Plants
------------	---------------------	--------

(% Individuals)		
T1	0	50- MS, 50-LS
T2	33	50-MS, 50-LS
T3	66	50-MS, 50-LS
T4	100	50- MS, 50-LS
T5	133	50-MS, 50-LS
T6 (Control)	100	100- LS

4.3. Plant Nutrition

Table 2 summarizes the nutrient solution formulations corresponding to each nitrate proportion used in the experiment. All solutions were prepared in 50 L containers, and the nutrient composition was carefully adjusted to maintain cationic balance [65], ensuring that variations in nitrate levels did not alter the electrical neutrality of the solution.

Table 5. Distribution of treatments and relative nitrate (NO_3^-) supply used in each experimental group.

Ions (mEq L ⁻¹)		Treatments.				
		T1	T2	T3	T4-T6	T5
MACRONUTRIENTS	KNO ₃	0.00	0.00	0.00	3.00	6.50
	Ca(NO ₃) ₂	0.00	4.00	8.00	9.00	9.00
	HNO ₃	0.00	0.90	0.90	0.90	0.90
	KH ₂ PO ₄	7.00	6.50	4.50	1.00	0.50
	K ₂ SO ₄	0.00	0.50	2.50	3.00	0.00
	CaSO ₄	9.00	5.00	1.00	0.00	0.00
	MgSO ₄ ·7H ₂ O	4.00	4.00	4.00	4.00	3.50
	H ₂ SO ₄	0.90	0.00	0.00	0.00	0.00
MICRONUTRIENTS	FeSO ₄	0.1430	0.1430	0.1430	0.1430	0.1430
	CuSO ₄	0.0194	0.0194	0.0194	0.0194	0.0194
	MnSO ₄	0.0031	0.0031	0.0031	0.0031	0.0031
	ZnSO ₄	0.0100	0.0100	0.0100	0.0100	0.0100
	Na ₂ [B ₄ O ₅ (OH) ₄]	0.0065	0.0065	0.0065	0.0065	0.0065
	Na ₂ MoO ₄	0.0030	0.0030	0.0030	0.0030	0.0030

4.4. Plant Growing Conditions.

An automatic Vertical Farm (VF) utilizing Internet of Things (IoT) technology has been established within the Artificial Illumination Laboratory (AIL) at TecNM | Campus Pabellón de Arteaga, Aguascalientes. The VF employs a multilevel Nutrient Film Technique (NFT) hydroponic system, designed in a rack measuring 1.955 m in width, 0.609 m in depth, and 1.981 m in height, located inside a cold room. Each level of the system contains three troughs, each 1.8 m long, with fifteen holes evenly spaced for placing the plants. The experiment involved three replicates, with five low-stress (LS) and five medium-stress (MS) plants for each of the treatments (T1, T2, T3, T4, T5), and ten LS plants for the control group (T6).

Nutrient solutions were stored independently in each gutter. A submersible pump facilitated the transfer of this solution through a Half-inch CPVC pipe. The flow was regulated by a five-eighths inch PVC hydraulic valve, maintaining a 1 % slope for the gutters and a water flow rate of 2.0 ± 0.2 liters per minute. The temperature was set at 20 ± 2 °C, with a relative humidity of $60 \pm 10\%$, and the carbon dioxide (CO₂) concentration was established at $1,100 \pm 300$ ppm.

4.4.1. Light System Characteristics

The artificial light environment was created using a combination of two LED light sources. One white LED produced a photosynthetic photon flux density (PPFD) of 200 ± 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while a blue LED provided a PPFD of 60 ± 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The PPFD was measured with a LightScout Solar Electric Quantum Meter from Spectrum Technologies, Inc. The emission spectra of the LEDs are illustrated in Figure 2.

These light spectra were recorded using a RedTide spectrophotometer from Ocean Optics. The photoperiod was set to 16 hours per day, and the total PPFD achieved was 260 ± 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$, resulting in a Daily Light Integral (DLI) of 14.976 ± 2.3 mol photons $\text{m}^{-2} \text{d}^{-1}$ [32].

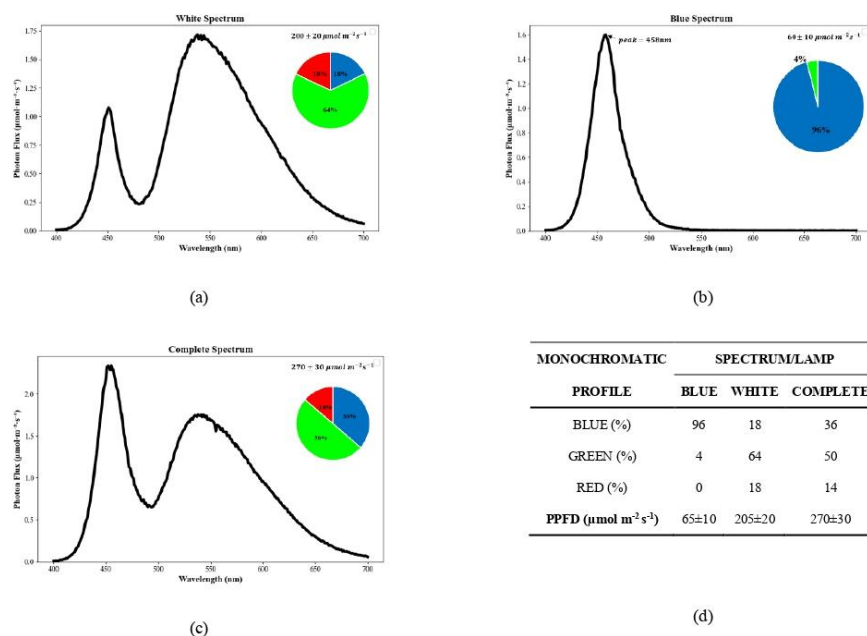


Figure 4. Distribution of Photosynthetic Photon Flux Density (PPFD) across different lighting treatments: (a) White LED lamps; (b) Blue LED lamps; (c) Both light sources combined; (d) Summary of spectral composition and proportions of light recipes for each spectrum. All values are expressed in nanometers (nm) within the electromagnetic spectrum.

4.5. Measurements of Plant Growth Parameters

4.5.1. Leaf Area (LA) and Number of Leaves (NL)

Leaf area (LA) was determined through digital image analysis utilizing ImageJ software version 1.54g. On day 30 of the treatment, the leaf blades from selected plants were

separated and photographed. Images were captured using an Apple iPhone 14 Pro equipped with a 48 MP wide-angle camera (f/1.78 aperture, Sony sensor; Apple Inc., USA), at a distance of 1 meter. Additionally, the leaves were categorized into three groups: Small, Medium, and Large, based on the largest and smallest leaf areas observed across all treatments.

4.5.2. Aerial Fresh and Dry Weight (AFW, ADW)

The Aerial Fresh Weight (AFW) was measured by weighing the root and stem separately using an OHAUS Scout® Balance. After weighing, these samples were placed in paper bags and dried in a forced convection oven (BINDER Drying Oven FD53) at 70°C for 72 hours. Once dried, the samples were weighed again.

4.5.3. Elemental Nutrient Analysis.

For the analysis of plant tissue nutrients, samples were dried, packaged in kraft paper bags, and sent to Fertilib Agricultural Laboratory located in Cd. Industrial de Celaya, Guanajuato, Mexico 38010. The laboratory is accredited as "Laboratorio de Ensayo EMA (SA-1359-044/21)." The analysis included the determination of total nitrogen using the Dumas method (accredited). Additionally, the elemental concentrations of boron (B), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), and nickel (Ni) were measured through acid digestion followed by ICP-OES analysis.

4.5.4. Specific Leaf Mass (SLM) and Specific Leaf Area (SLA)

Equation 1 was used to calculate Specific Leaf Mass (SLM), based on leaf dry mass and leaf area, following the method described by Mickens et al., [30].

$$SLM = \frac{M_{dr}}{LA} \quad (1)$$

SLM stands for specific leaf mass per area (mg/cm²), M_{dr} indicates the aerial dry weight (mg), and LA represents leaf area (cm²).

4.5.5. Water Use Efficiency (WUE)

Equation 2 presents the calculation of Water Use Efficiency (WUE), a key indicator of resource utilization under controlled environmental conditions. WUE was determined by dividing the total Plant Fresh Weight (g FW) by the volume of water used (L H₂O) during the crop cycle:

$$WUE = \frac{\text{Plant Fresh Weight (g FW)}}{\text{Water Volume Used (L H}_2\text{O)} - \frac{\text{COLLECTED WATER (L H}_2\text{O)}}{6}} \quad (2)$$

4.6. Measurements of water consumption (WC), pH and electrical conductivity (EC)

4.6.1. Water consumption (WC)

Water consumption was monitored weekly throughout the experiment. Each treatment used 50-liter capacity basins, which were initially filled with 47.5 liters of water. To track the remaining water volume over time, a simulation was designed in Python using the geometric dimensions of the basin. This approach established a direct relationship between the liquid level (height) and the actual volume, allowing for accurate estimation of weekly water consumption based on changes in height.

4.6.2. pH and EC

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The pH and electrical conductivity (EC) were measured daily for all six nutrient solutions before the start of the daily light integral (DLI) using an AQUASEARCHER™ AB33M1-F benchtop meter (OHAUS, US). The target pH value was set within the range of 6.0 ± 0.5 . If necessary, pH adjustments were made by adding nitric acid or sulfuric acid, depending on the specific treatment, to avoid altering the nitrate (NO_3^-) concentration. Sodium hydroxide ACS (Fermont) was used when it was necessary to increase the pH. The target EC value was set between 1.8 and 2.5 mS cm^{-2} , and no adjustments to the EC were required throughout the experiment.

4.7. Energy Consumption

For each treatment, energy consumption was measured using a STEREN electricity consumption meter (Wattmeter-W-) HER-432, located in Tlalnepantla de Baz, State of Mexico, Mexico. This equipment operates at 120 V AC and a frequency of 60 Hz, with a minimum current measurement of 1mA up to 15A. The power consumption readings are independent of the measured values. Electricity consumption was divided into four main categories: lamps, pumps, CO_2 systems, and a mini-split HVAC system. Additionally, consumption from the automated environment, which includes a Raspberry Pi 4B, Arduino, ESP32CAM, sensors, and various actuators, was also measured using the same STEREN electricity consumption meter.

4.8. Statical Analysis.

An analysis of variance (ANOVA) was conducted to identify significant differences among treatments regarding various physiological and growth-related variables. When significant differences were found ($p < 0.05$), Tukey's HSD (Honestly Significant Difference) test was used for mean comparisons between treatments, maintaining a significance level of 5% (95% confidence). All statistical analyses were performed using MINITAB 20 software, with a confidence level of 95% ($\alpha = 0.05$).

5. Conclusions

This study presents strong evidence that carefully adjusting nitrate (NO_3^-) concentration in vertical farming NFT (Nutrient Film Technique) systems, along with implementing agroecological practices such as intercropping with *Medicago sativa* and inoculating with *Rhizobium*, significantly improves the yield, physiological efficiency, and nutritional quality of *Lactuca sativa* (lettuce). The results showed that an intermediate nitrate level (designated as T3) optimized shoot biomass production, reduced nitrate accumulation in edible tissues by 29 %, and enhanced water use efficiency by 38 % compared to monoculture systems. Additionally, the symbiotic relationship with *M. sativa* improved rhizospheric conditions, decreased electrical conductivity, and facilitated the uptake of essential micronutrients like zinc (Zn) and manganese (Mn), helping to counteract the negative effects of excess NO_3^- .

The findings highlight the potential for integrating biotechnological tools and ecological strategies in highly controlled environments like vertical farming. This approach creates a new model of sustainable intensification that emphasizes not only yield but also nutritional efficiency and food safety, with significant implications for reducing nitrate accumulation in fresh produce.

Building on these results, future research should incorporate transcriptomic and metabolomic analyses to identify the molecular pathways involved in nitrogen assimilation, especially under crop association conditions. It is also recommended to assess the effects of various LED spectral compositions (e.g., 80 % Red and 20 % Blue) in conjunction with CO_2 supplementation and different Daily Light Integral (DLI) values on the gene expression of key enzymes, such as nitrate reductase and glutamine synthetase. These

research directions will provide a comprehensive understanding of how nutrition, light, plant physiology, and microbial symbiosis interact in vertical farming environments.

Finally, the postharvest phase should be investigated, taking into account nutraceutical parameters, shelf life, and microbial safety. This will contribute to a complete and sustainable vision for the future of urban agriculture.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/doi/s1>, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.” Please turn to the [CRediT taxonomy](#) for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section “MDPI Research Data Policies” at <https://www.mdpi.com/ethics>.

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Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

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APPENDICE 2

Title Op1: "Biomechatronic Regulation and Quantum Efficiency (ϕ PSII)-Based Performance Analysis of Lettuce in Association with Alfalfa in NFT Vertical Farming"

Title Op2: "Biomechatronic Regulation and ϕ PSII Quantum Efficiency Assessment in Lettuce–Alfalfa Associations under Vertical NFT Cultivation"

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0. Abstract:

Integration of physiological monitoring into biomechatronic systems represents a novel approach to optimize energy use and photosynthetic performance in controlled-environment agriculture. In this study, we evaluated the effect of biomechatronic regulation and quantum efficiency (ϕ PSII) monitoring on the growth and energy use efficiency of *Lactuca sativa* cultivated in association with *Medicago sativa* under a Nutrient Film Technique (NFT) vertical farming system. Lettuce seedlings were co-cultivated with alfalfa under six different nitrate regimes, with strict control of environmental variables including temperature, humidity, CO₂ concentration, and photosynthetically active radiation. Physiological indicators such as chlorophyll content (SPAD), light use efficiency (LUE), energy use efficiency (EUE), effective quantum yield of PSII ($Y(II)$), and non-photochemical quenching (NPQ) were monitored weekly. Results demonstrated that moderate nitrogen supplementation (T3) combined with alfalfa association enhanced photosynthetic

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performance ($Y(II) = 0.7467$, $Fv/Fm = 0.7591$) while maintaining low stress levels ($NPQ < 0.01$) and high resource use efficiencies ($LUE = 0.82 \text{ g DW mol}^{-1}$; $EUE = 1.37 \text{ g FW kWh}^{-1}$). Excessive or deficient nitrogen negatively affects physiological parameters and energy performance. The proposed biomechatronic system ensured stable microenvironmental conditions and enabled dynamic insights into plant physiology, presenting a scalable model for sustainable vertical farming with reduced fertilizer input and improved energy efficiency.

Keywords: Biomechatronic, Quantum Efficiency ($\phi PSII$), Chlorophyll, Light Use Efficiency (LUE), Energy Use Efficiency (EUE), Nutrient Film Technique (NFT), Vertical Farming.

1. INTRODUCTION.

Sustained growth of the global population, the effects of climate change, and accelerated urbanization have generated unprecedented pressure on traditional agricultural systems. In response to this scenario, controlled-environment agriculture (CEA) has emerged as a viable technological alternative for intensive and sustainable food production. This approach allows crops to be isolated from external climatic variations, improves the use of water, energy, and nutrients, and enables precise adjustment of environmental variables that directly affect plant physiology [1–3]

In this context, biomechatronic systems have gained prominence for their ability to integrate sensors, actuators, microcontrollers, and IoT platforms that automatically regulate key parameters such as temperature, humidity, carbon dioxide (CO_2) concentration, light intensity, and fertigation. The use of open-source controllers like ESP32, Arduino, and Raspberry Pi has democratized the design of intelligent agricultural solutions, supported by mobile applications and real-time visualization

platforms [4–6]. Various studies have reported significant improvements in the operational efficiency of automated hydroponic systems. [7,8] documented biomass increases of up to 30% and significant water savings thanks to automated control of variables such as pH (5.5–6.5), EC (1.5–2.2 mS/cm), temperature (23–26 °C), and humidity (60–75%).

Similarly, systems such as the one proposed by [9] have incorporated CO₂ and PPFD sensors to enhance atmospheric and lighting control. However, these advances share a critical limitation: the lack of feedback based on the actual physiological status of the plants. Currently, most systems rely on the control of external environmental variables without considering the biophysical responses of crops as inputs for decision-making. This disconnect prevents the dynamic adaptation of the environment to the metabolic needs of the crop, thereby limiting potential efficiency. One of the most promising approaches to overcome this barrier is the physiological monitoring of photosystem II (PSII), responsible for the light-dependent phase of photosynthesis. Parameters such as Fv/Fm (maximum quantum yield), Y(II) (operational efficiency), and NPQ (non-photochemical quenching) are recognized as key indicators for detecting light stress, energy capture efficiency, and photoprotection mechanisms [10,11].

[12] correlated Fv/Fm' with the rate of CO₂ assimilation and yield in wheat, while [13] reported NPQ increases from 0.2 to 1.2 under water stress conditions. The use of fluorometric sensors like the JUNIOR-PAM allows these parameters to be recorded non-destructively and in real time, enabling the design of cyber-physical systems with adaptive control based on plant physiology. [14] demonstrated that integrating NPQ and ΦPSII into spectral lighting control improves the energy balance of horticultural crops. In parallel, efficiency indicators such as light use efficiency (LUE, g DW mol⁻¹) and energy use efficiency (EUE, g FW kWh⁻¹) are

essential for evaluating sustainability in vertical agriculture. According to [15–17], typical LUE values for *Lactuca sativa* range from 0.56 to 0.75 g DW mol⁻¹, while the average EUE remains below 1.2 g FW kWh⁻¹. [18] indicated that optimizing PPFD between 250 and 300 μmol m⁻² s⁻¹ can raise EUE to up to 1.4 g FW kWh⁻¹. However, these indicators are often analyzed in isolation without integration with dynamic physiological variables.

Additionally, the use of interspecific symbiosis, such as the association between *Lactuca sativa* and *Medicago sativa*, offers an agroecological strategy to improve nitrogen use efficiency [19]. This interaction reduces dependence on synthetic fertilizers, increases nitrogen bioavailability through biological fixation, and enhances the expression of photosynthetic genes [20].

Nonetheless, the interaction between this symbiosis, photosynthetic physiology, and light efficiency has not yet been explored under automated controlled conditions. The current literature reveals a gap characterized by the lack of integration between environmental monitoring, photosynthetic physiological analysis, and adaptive regulation of cultivation variables. This disconnection limits the real efficiency of automated vertical farming systems and represents an opportunity for developing cyber-physical solutions with greater adaptability and sustainability. Therefore, the objective of this research is to evaluate the impact of integrated environmental and physiological monitoring of photosystem II (PSII), combined with the symbiotic association with *Medicago sativa*, on the photosynthetic, energetic, and nutritional efficiency of *Lactuca sativa* within an automated hydroponic vertical farming system.

2. Materials and Methods

2.1 Plant Material and Experimental Design

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Lactuca sativa L. longifolia (LS) seedlings, three weeks post-germination, were sourced from the Zapata Greenhouse (Asientos, Aguascalientes, Mexico). Following their acquisition, the seedlings were kept for an additional week under controlled conditions, exposed to continuous artificial light at an intensity of $160 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$. After completing four weeks of growth, they developed the necessary morphological traits for transplantation.

Simultaneously, *Medicago sativa* (MS), certified San Miguelito variety, characterized by a 90% germination rate and 98% purity, was cultivated. Seeds were sown in black plastic seedling trays with 60 cavities (one seed per cavity) and, during their development stage, were grown under the same environmental conditions and light intensity as *Lactuca sativa* during its final week, ensuring homogeneous acclimatization between the two species.

Subsequently, both species were transplanted together into a Nutrient Film Technique (NFT) system, configured as the experimental platform. Five nutrient solutions were formulated based on the Steiner (1984) scheme, modifying the nitrate concentration. Each solution maintained specific proportions of anions and cations, ensuring physiological compatibility between the two species. The baseline solution maintained an electrical conductivity (EC) of $2.0 \pm 0.5 \text{ mS cm}^{-1}$ and a pH of 6.0 ± 0.5 , parameters standardized across all treatments to ensure chemical uniformity in the root environment.

The experimental design included six treatments defined by variations in the nitrate (NO_3^-) concentration of the nutrient solution, aimed at evaluating the response of *Lactuca sativa* (LS) and *Medicago sativa* (MS) under association and different nitrogen supply regimes. Treatments T1, T2, T3, T4, and T5 contained relative NO_3^- concentrations of 0%, 33%, 66%, 100%, and 133%, respectively, with a constant ratio of 50% LS and 50% MS in each experimental unit. Treatment T6 was established as

an absolute control, composed exclusively of *Lactuca sativa* (100% individuals), grown under the standard solution with 100% NO_3^- .

This factorial configuration allowed not only the assessment of nitrogen supply effects on the growth and development of both species but also the identification of potential interspecific interactions in terms of competition or physiological synergy.

2.2 Biomechatronic Environment and Regulation of the Growing Environment.

2.2.1 Overview

The study was conducted in an automated vertical farm configured as a biomechatronic environment, designed to precisely regulate the microenvironmental conditions required for the physiological development of *Lactuca sativa* (LS) in association with *Medicago sativa* (MS).

The system integrated distributed sensors, actuators, and electronic modules capable of real-time monitoring and control of critical variables such as temperature, relative humidity, CO_2 concentration, nutrient solution flow, and light intensity.

All functions were coordinated by a central control unit, which automatically managed the various subsystems, including lighting, ventilation, irrigation, and CO_2 enrichment. This architecture ensured the stability and reproducibility of experimental conditions, providing a controlled environment essential for accurately evaluating the physiological responses of both species to different nutritional treatments.

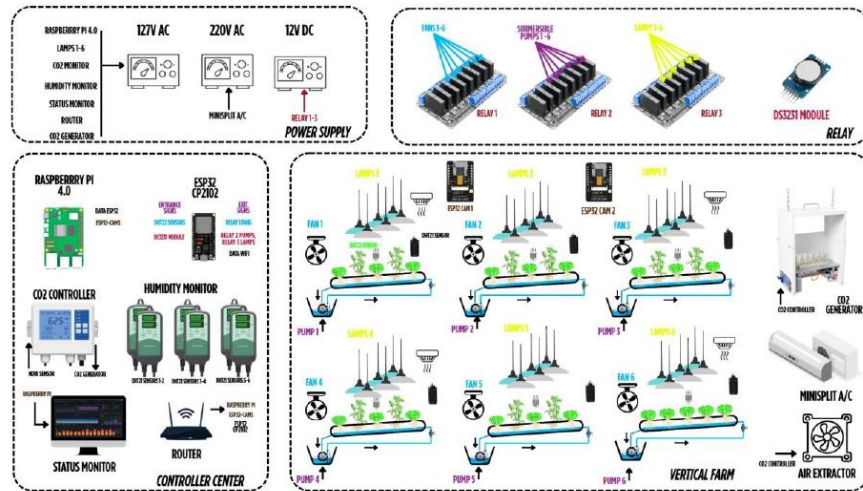


Figure 1. Diagram of the biomechatronic vertical farming system, showing the integration of sensors, actuators, and a control unit for real-time regulation of environmental variables.

Figure 2 illustrates the logical sequence of operation of the biomechatronic control system used in the vertical farming platform. The process begins with system activation and verification of Wi-Fi connectivity. Upon successful connection, a Raspberry Pi 4.0B unit communicates with multiple ESP32 modules responsible for acquiring data from environmental sensors and controlling corresponding actuators.

CO₂ levels are monitored through NDIR sensors, triggering a CO₂ generator when concentrations fall below 1000 ppm. Gas leaks and smoke are detected via MQ6 sensors, activating the air extractor if levels exceed 1300 ppm. Humidity and temperature are monitored by DHT21 sensors, which activate ventilation through relay-controlled fans if values exceed defined thresholds (e.g., >22°C or >70% RH).

Additionally, DHT22 sensors provide real-time data to regulate both temperature and humidity, while the DS3231 timing module manages light cycles and pump activation. Lighting control is executed through relay boards operating two lighting groups (white and blue LEDs), scheduled between 18:00 and 10:00. Climate stability is maintained by a minisplit system configured to 20°C and 65% relative humidity. The entire system is monitored remotely via a web server, with visual crop data collected by ESP32-CAM modules

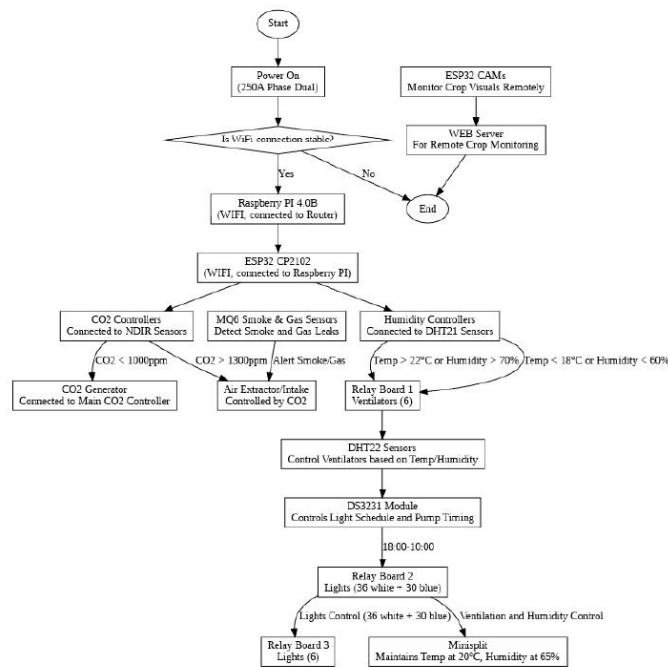


Figure 2. Sequence of operations for environmental monitoring and control in the vertical farming system, including CO₂, temperature, humidity, lighting, and ventilation regulation.

2.2.2 Temperature and Humidity

Thermal and humidity regulation was achieved using an automated system controlled by DHT21 and DHT22 sensors integrated into ESP32 microcontrollers connected to the central Raspberry Pi 4.0B unit. These sensors enabled real-time monitoring of the internal environmental conditions, triggering actuators based on predefined thresholds. When the temperature exceeded 22 °C or the relative humidity surpassed 70%, ventilation fans connected to Relay Board 1 were automatically activated to facilitate air exchange.

Under low-temperature conditions (<18 °C) or low humidity (<60%), the system activated corrective mechanisms, including the operation of the minisplit, which maintained a stable temperature of 20 °C and relative humidity of 65%. This configuration ensured constant environmental conditions suitable for the optimal growth and physiology of both plant species.

2.2.3 CO₂

Carbon dioxide (CO₂) concentration was monitored and regulated using three DANOPLUS AZ-7530 controllers (DANOPLUS, Taiwan), connected to high-precision NDIR sensors (Senseair, Sweden). These devices allowed continuous measurement of CO₂ levels within the cultivation environment, adjusting the concentration between 1000 and 1300 ppm to promote photosynthetic efficiency. When CO₂ concentrations fell below the target threshold, a VEVOR W4B-LP (VEVOR, China) CO₂ generator was automatically activated, increasing CO₂ levels to approximately 1250 ppm. In case of excessive accumulation (concentrations above 1300 ppm), the system activated air extraction and intake modules (intractor/extractor) to restore safe and physiologically optimal CO₂ levels. This

feedback mechanism maintained stable atmospheric conditions throughout the experimental period.

2.2.4 Artificial Lighting

The lighting system consisted of an LED configuration with an 80W20B spectral recipe, composed of 80% white light and 20% blue light. In each treatment, five TISHMAN TLT83665CLV 36 W, 6500 K LED tubes (TISHMAN, Mexico) and six T8 18 W LED tubes (Philips, Netherlands) were strategically installed to ensure uniform irradiance over the crops.

Photosynthetic photon flux density (PPFD) was $200 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$ for white light and $60 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ for blue light, measured using a Solar Electric Quantum Meter (Solar Light Co., USA). The combined spectrum was validated using an Ocean Optics RedTide USB650 spectrometer (Ocean Insight, USA) (see Figure 3). A photoperiod of 16 hours of light and 8 hours of darkness was established, achieving a total PPFD of $260 \pm 40 \mu\text{mol m}^{-2} \text{s}^{-1}$ across the cultivation surface. Lighting activation and shutdown were managed by a DS3231 real-time clock module (Maxim Integrated, USA) connected to six solid-state relays (Omron, Japan), ensuring a constant and reproducible lighting regime throughout the experimental cycle.

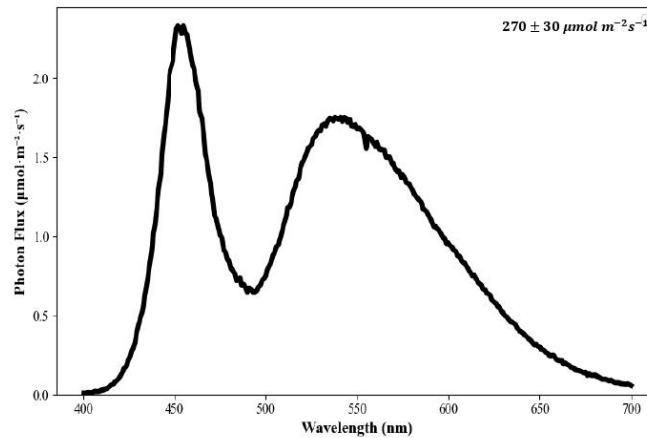


Figure 3. Spectral distribution of the LED lighting system with a total PPFD of $270 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$ under the 80W20B light recipe.

Figure 4 shows a heat map of the spatial distribution of irradiance (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) across each of the treatments (T1 to T6) applied within the cultivation system. Each square represents the position of a plant within the experimental area, and the numerical value indicates the light intensity measured at that point. The colors range from blue (low intensity) to dark red (high intensity), according to the scale located on the right side of the image. Most positions exhibit levels above $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, suggesting an overall homogeneous light distribution, with slight variations observed at the edges and central zones in some treatments. The deep blue areas indicate regions without direct light exposure, corresponding to aisles or gaps between cultivation modules.

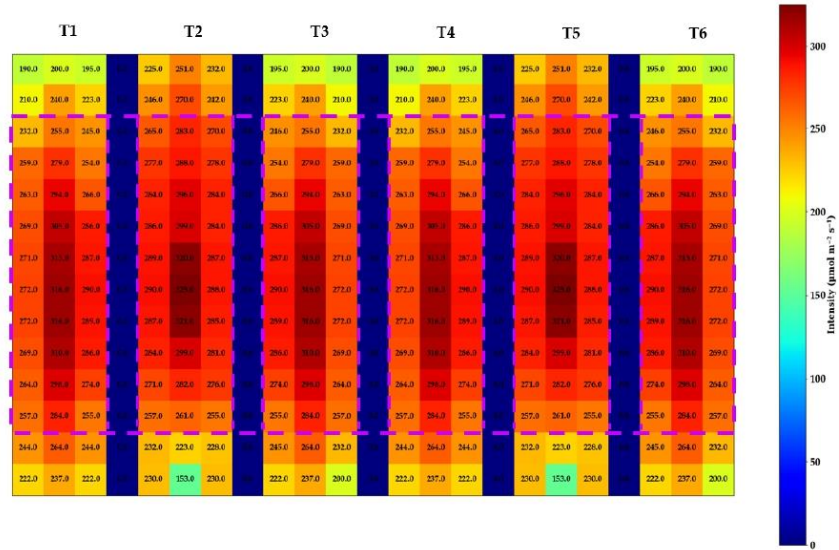


Figure 4. The spatial distribution and uniformity of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) across all treatments (T1–T6) within the cultivation system.

2.2.5 Irrigation System

The automated irrigation system operated with a regulated flow rate between 1 and 2 liters per minute, using submersible water pumps (Kulife, USA) connected to a central control system. The pumps were activated in scheduled cycles of four hours of continuous operation, followed by 20-minute rest intervals. This configuration ensured constant and controlled circulation of the nutrient solution within the NFT system, guaranteeing homogeneous distribution across all cultivation units.

2.2.6 Connections and Monitoring

The entire biomechatronic system was interconnected through an ESP32 CH340C microcontroller (Espressif Systems, China), which communicated with a central Raspberry Pi 4 Model B unit (Raspberry Pi Foundation, United Kingdom) via a local

Wi-Fi network. This architecture enabled real-time data collection from distributed sensors, as well as visualization and analysis using the Grafana platform (Grafana Labs, USA). Additionally, two ESP32-CAM units (Espressif Systems, China) were integrated into the Raspberry Pi, allowing remote visual monitoring of the system and crop status throughout the experimental period.

2.3 Plant Nutrition

The nutrient solutions differed solely in nitrate ion (NO_3^-) concentration, set at 0%, 33%, 66%, 100%, and 133% for treatments T1 to T5, respectively, along with a control treatment (T6) formulated with the same NO_3^- level as T4 (100%). The corresponding NO_3^- concentrations for each solution were 0, 4, 8, 12, and 16 mEq L^{-1} , respectively, while both T4 and the control shared the standard Steiner (1984) solution, containing 12 mEq L^{-1} of NO_3^- . All solutions were adjusted to maintain constant concentrations of other anions and cations, as well as a pH of 6.0 ± 0.5 and electrical conductivity of $2.1 \pm 0.7 \text{ mS cm}^{-1}$, allowing for an isolated evaluation of nitrate's effects on the physiological responses of *Lactuca sativa* and *Medicago sativa*.

2.4 Chlorophyll, EUE and LUE Measurements

Chlorophyll content was evaluated weekly through readings taken at three specific moments of the photoperiod: one hour before the beginning of the light cycle, halfway through the photoperiod, and one hour before its end.

Measurements were made using a portable SPAD-502 meter (Minolta, Japan), referencing the middle third of the leaf blade, following the methodology described by Reis et al. (2006).

Chlorophyll values were expressed in SPAD units, enabling non-destructive evaluation of relative pigment concentration variations throughout the experiment.

Energy Use Efficiency (EUE) was calculated following the methodology proposed by Pennisi et al. (2020), considering the total fresh weight (FW) of leaves obtained at the end of the experiment. The total electrical consumption of the lighting system was recorded, expressed in kilowatt-hours (kWh). This indicator allowed quantification of system efficiency in terms of fresh biomass produced per unit of energy consumed. The EUE value was determined according to Equation 1.

$$EUE = \frac{\text{Final Leaf Fresh Weight (g FW)}}{\text{Electricity Consumption (kWh)}} \quad (1)$$

Light Use Efficiency (LUE) was calculated based on the definition established by Pennisi et al. (2020), considering the efficiency of converting absorbed photosynthetically active radiation (APAR) into accumulated dry biomass (Dry Biomass, DB). Unlike classical approaches based on Gross Primary Productivity (GPP), this formulation focused on agronomic metrics applicable to performance evaluation in controlled-environment cultivation systems. This parameter assessed physiological crop efficiency as a function of absorbed radiation and dry biomass production during the experiment. Equation 2 represents this relationship.

$$LUE = \frac{\text{Dry Biomass (g m}^{-2}\text{)}}{\text{APAR (MJ m}^{-2}\text{)}} \quad (2)$$

2.5 Photosynthetic Efficiency Evaluation (PSII)

The photosynthetic efficiency of Photosystem II (ϕPSII) was evaluated weekly using a JUNIOR-PAM chlorophyll fluorometer (Walz, Germany). Measurements were taken from fully expanded leaves selected from the middle third of each plant using an open leaf clip placed over the leaf blade. Two light-adapted and three dark-adapted measurements were performed at different times during the photoperiod.

For light-adapted measurements, the clip remained open to allow ambient illumination, and steady-state fluorescence (F_s) was recorded for at least one minute. Then, a saturating light pulse was applied to obtain the maximum fluorescence under light (F_m'). Subsequently, without removing the clip, the sample was completely covered and kept in darkness for 10 minutes to allow photosynthetic apparatus relaxation. Under these dark conditions, minimum fluorescence (F_o) and maximum fluorescence (F_m) were recorded using a second saturating pulse. Measurements were taken at 8 and 16 hours after the beginning of the daily light interval (DLI) for light- and dark-adapted protocols. An additional dark-adapted measurement was conducted two hours before the start of the photoperiod (-2h DLI) to capture the basal physiological state prior to light exposure.

Three key photosynthetic efficiency parameters were calculated from the data: Effective quantum yield of PSII ($Y(II)$), calculated as $(F_m' - F_s) / F_m'$; Maximum quantum yield of PSII (F_v/F_m), calculated as $(F_m - F_o) / F_m$; Non-photochemical quenching (NPQ), calculated as $(F_m - F_m') / F_m'$.

These indicators reflect the functional status of the photosynthetic apparatus and the physiological responses of plant species to different nutritional and light treatments.

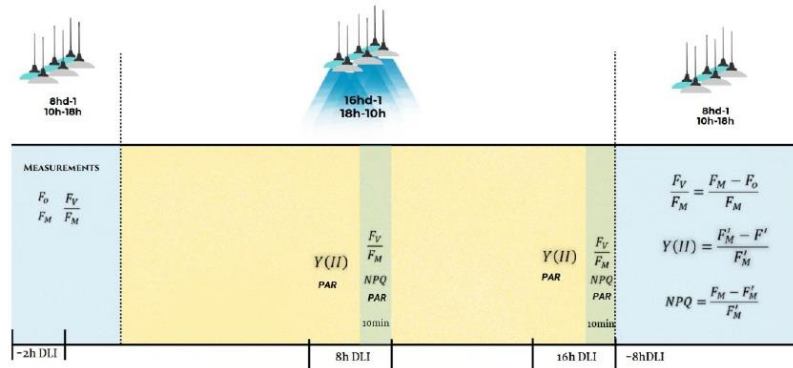


Figure 5. Workflow for the measurement of effective quantum yield ($Y(II)$), maximum quantum efficiency (F_v/F_m), and non-photochemical quenching (NPQ) of PSII in *Lactuca sativa*.

2.6 Energy Consumption

Electrical consumption for each treatment was recorded using a STEREN HER-432 meter (Mexico), operating at 120 V AC and 60 Hz, with a measurement range of 1 mA to 15 A. The analysis considered four main categories: lighting (white and blue lamps), irrigation (submersible pumps), environmental control (Drummond MSDFE12HP-3, BOHN ADT130UC, INKBIRD IHC-200, extractor, intractor, and fans), and automation (Raspberry Pi 4B, ESP32, ESP32-CAM, sensors, actuators, router, status monitor, and regulated power supply). The CO₂ enrichment system (DANOPLUS AZ-7530 controller and generator) was also included. This classification allowed quantification of specific consumption by subsystem within the automated cultivation environment.

2.7 Statistical Analysis

The effect of the association between *Medicago sativa* (MS) and *Lactuca sativa* (LS) under different nitrate concentrations was evaluated through analysis of variance (ANOVA). The experimental design included five association treatments (0%, 33%, 66%, 100%, and 133% NO_3^-), each with five LS and five MS plants, plus a control group consisting of ten LS monoculture plants, totaling 180 plants (105 LS and 75 MS). To identify significant differences ($p < 0.05$) among treatments for physiological and growth variables, ANOVA was followed by Fisher's Least Significant Difference (LSD) test for mean comparisons. Additionally, correlation and regression analyses were conducted to determine relationships between growth, nutrition, and photosynthetic efficiency variables. All statistical analyses were performed using MINITAB 20 software, with a confidence level of 95% ($\alpha = 0.05$).

3. Results.

3.1 Evaluation of Environmental Conditions and Crop Response

The biomechatronic system maintained a stable environmental setting that favored the development of *Lactuca sativa* and *Medicago sativa*. Thermal regulation remained within the physiologically optimal range, averaging 20.54 ± 1.12 °C, thus avoiding fluctuations that could affect metabolic processes. Simultaneously, relative humidity was controlled at $57.67 \pm 4.12\%$, promoting efficient transpiration and proper tissue hydration. Additionally, CO_2 concentration stabilized at 1078.56 ± 129.56 ppm, ensuring continuous availability for photosynthesis without reaching saturation levels that could compromise efficiency.

Light distribution was homogeneous, with variations below 10% across positions, ensuring uniform irradiance over the plant canopy and minimizing differential radiation use among plants. Irrigation supply was delivered at a constant flow of 1.8 to 2.0 L/min, in cycles of four hours of continuous operation followed by 20-minute

resting intervals, maintaining adequate water availability without causing root zone flooding. The NFT system's inclination, adjusted between 1.5% and 2%, promoted a continuous laminar flow of the nutrient solution, improving root oxygenation and preventing localized accumulations.

3.2 Chlorophyll Accumulation Dynamics in *Lactuca sativa*.

The evolution of chlorophyll content, expressed in SPAD units, showed significant differences between treatments and evaluation times (Table 1). At the beginning of the experiment (day 0), no differences between treatments were observed, registering a uniform value of 17 ± 0.00 SPAD in all groups. During the first week, treatments T1, T3 and T5 presented significantly higher values ($p < 0.05$) compared to T2, T4 and T6, suggesting a differentiated early response to fertilization or plant association treatments. In the second week, a generalized increase in chlorophyll content was observed in all treatments, with no significant differences detected among them. From the third week on, clear differences emerged: treatment T6 recorded the highest value (41.01 ± 8.52 SPAD), followed by T3, T5 and T4, which showed intermediate values. In the fourth week, treatments T3, T4, T5 and T6 maintained the highest chlorophyll contents, significantly exceeding treatments T1 and T2. At the end of the experiment, the highest chlorophyll content was recorded in T5 (53.20 ± 9.66 SPAD), followed by T3 (51.16 ± 4.18 SPAD), T4 (50.88 ± 4.29 SPAD) and T6 (50.07 ± 2.51 SPAD). In contrast, T1 and T2 presented the lowest values, which could be associated with limitations in nitrogen availability or the absence of symbiotic interaction.

Table 1. Chlorophyll content (SPAD units) measured weekly in *Lactuca sativa* under different nitrate treatments. Values represent mean $\pm$ standard deviation. Different letters within the same column indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$).

Treatments	SPAD units				
	Day 0	W1	W2	W3	W4
T1	17 ± 0.00 ^a	20.67 ± 0.51 ^a	28.12 ± 4.47 ^a	29.85 ± 2.25 ^c	37.16 ± 2.47 ^b
T2	17 ± 0.00 ^a	17.23 ± 1.94 ^b	27.45 ± 3.88 ^a	33.92 ± 3.40 ^{bc}	38.30 ± 2.19 ^b
T3	17 ± 0.00 ^a	21.07 ± 0.51 ^a	33.22 ± 4.40 ^a	37.61 ± 3.90 ^{ab}	51.16 ± 4.18 ^a
T4	17 ± 0.00 ^a	18.10 ± 0.51 ^b	30.76 ± 6.73 ^a	35.81 ± 5.21 ^{ab}	50.88 ± 4.29 ^a
T5	17 ± 0.00 ^a	21.67 ± 0.51 ^a	29.31 ± 6.75 ^a	37.48 ± 2.65 ^{ab}	53.20 ± 9.66 ^a
T6	17 ± 0.00 ^a	17.27 ± 1.26 ^b	30.34 ± 4.98 ^a	41.01 ± 8.52 ^a	50.07 ± 2.51 ^a

3.2.1 LUE y EUE

Figure 6 represents the Light Use Efficiency (LUE) values presented remarkable differences between treatments. The treatment with 66 % nitrogen showed the highest efficiency, with a value of 0.82 g DW mol⁻¹, indicating a more effective utilization of photosynthetically active radiation. Interestingly, the control treatment (without inoculation of *Medicago sativa*) reached an even higher value (0.90 g DW mol⁻¹), with no evidence of a reduction attributable to the absence of legume symbiosis. In contrast, the treatment with 0 % nitrogen presented the lowest value (0.46 g DW mol⁻¹), reflecting a clear nutritional limitation that affected photosynthetic efficiency. The 100% and 133% nitrogen treatments did not significantly exceed the 66% nitrogen treatment, suggesting that high nitrate levels do not improve light efficiency, possibly due to saturation or metabolic dilution effects.

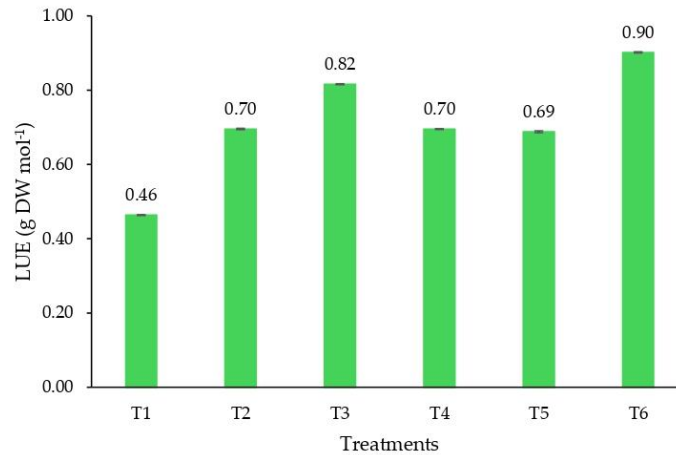


Figura 6. Energy use efficiency (g FW kWh⁻¹) in *Lactuca sativa* under different nitrate treatments. Error bars represent standard deviation.

Electricity Use Efficiency (EUE) showed marked differences between treatments (Figure 7). The control treatment registered the highest value (1.50 g FW kWh⁻¹), followed by the 66 % nitrogen treatment (1.37 g FW kWh⁻¹), reflecting a more efficient conversion of the electrical energy consumed into fresh biomass. In contrast, the 0 % nitrogen treatment showed the lowest energy efficiency (0.23 g FW kWh⁻¹), indicating severe limitations in plant growth due to nitrogen deficiency. Similar to what was observed in LUE, the treatments with higher nitrate concentrations (100 % and 133 %) did not show significant improvements with respect to the 66 % treatment, suggesting the existence of a saturation threshold in the productive response to increases in nitrogen availability.

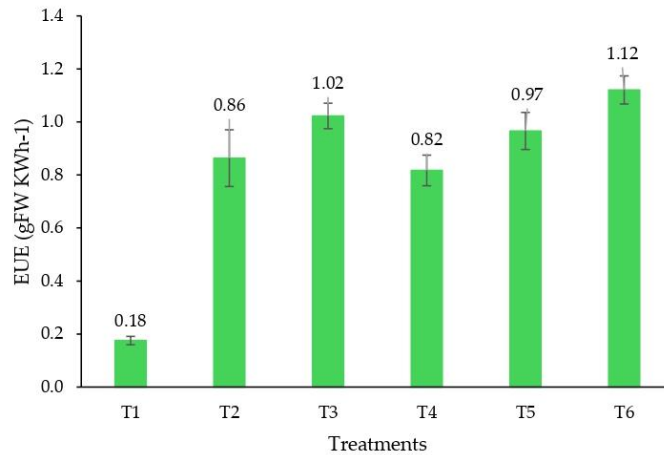


Figure 7. Energy use efficiency (g FW kWh⁻¹) in *Lactuca sativa* under different nitrate treatments. Error bars represent standard deviation.

3.3 Photosynthetic Performance. Quantum Yield of PSII [Y(II)]

The quantum efficiency of photosystem II [Y(II)] was evaluated at 8 and 16 h of the exposure period to photosynthetically active radiation for four weeks (Figure 8). In general, the values recorded at 8 h were higher than those measured at 16 h, indicating a progressive reduction of photosynthetic efficiency towards the end of the daily illumination period. In the treatment with 0 % nitrogen (T1), associated with *Medicago sativa*, Y(II) showed a slight decrease over time, going from 0.712 in week 1 to 0.683 in week 4 at 8 h, while at 16 h the values remained relatively stable, between 0.617 and 0.632. The treatment with 33 % N (T2) presented greater stability, with minimal variations between weeks both at 8 h and 16 h. On the other hand, the treatment with 66 % nitrogen (T3) registered the highest Y(II) values, reaching a maximum of 0.747 at 8 h in week 4, and remaining around 0.715 at 16 h, standing out for its high temporal stability. The treatment with 100 % N (T4) showed an

increasing trend, going from 0.7020 to 0.7347 at 8 h between week 1 and 4, and from 0.6877 to 0.7098 at 16 h. In contrast, the treatment with 133 % N (T5) evidenced a reduction in efficiency at week 4, registering values of 0.6913 at 8 h and 0.6672 at 16 h. Finally, the control treatment (T6), without association with *Medicago sativa*, maintained relatively constant values throughout the experiment, although with a slight decrease at the end (from 0.6997 at 8 h and 0.6722 at 16 h).

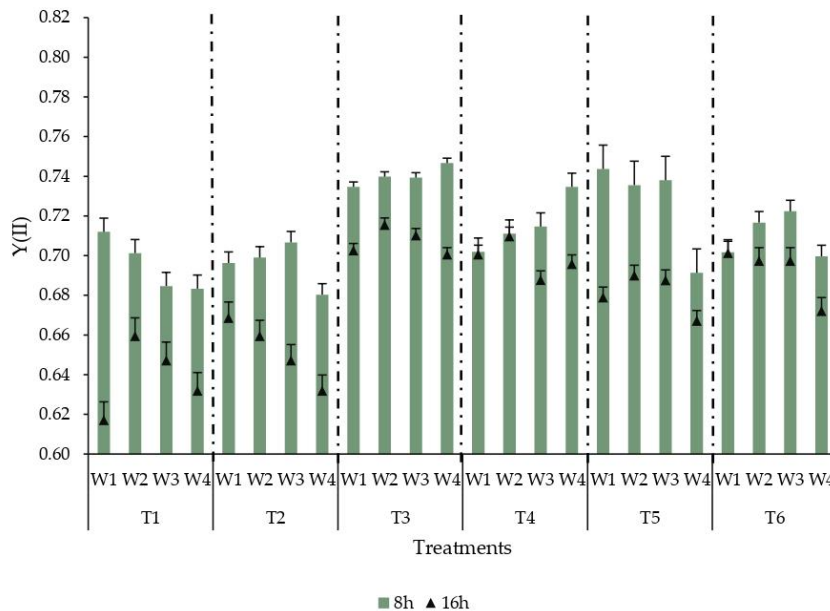


Figure 8. Effective quantum yield of ϕPSII [$Y(\text{II})$] in *Lactuca sativa* under different nitrate treatments, measured at 8 h and 16 h over four weeks. Error bars represent standard deviation.

3.4 Photoprotection and Light Stress-Maximum Quantum Efficiency of PSII

The maximum quantum efficiency of photosystem II [F_v/F_m] was evaluated at -2, 8 and 16 h of the light exposure period for four weeks, under conditions of association

with *Medicago sativa*, except in the control treatment. Values obtained two hours before the onset of the photoperiod (-2) remained stable, between 0.75 and 0.79 in most treatments, reflecting an adequate basal efficiency of PSII. However, in week 4, the 0 % N treatment recorded a reduction to 0.7500, indicating a functional weakening of the photosystem in the absence of fertilization. Fv/Fm values evaluated at 8 h, after a brief dark adaptation period, showed clear differences between treatments. The treatment with 66 % nitrogen reached the highest value at week 4 (0.7511), while the treatment with 0 % N presented the lowest value (0.6858), showing a lower photosynthetic recovery capacity under nitrogen restriction. At 16 h, a generalized decrease was observed in all treatments, attributable to accumulated light stress after prolonged exposure to radiation. Despite this reduction, the 66 % N treatment and the control maintained the highest values at week 4 (0.7256 and 0.7421, respectively), indicating greater functional stability of PSII in the face of stress. In contrast, the 133 % N treatment showed a remarkable drop to 0.6930, suggesting a lower efficiency of photoprotection mechanisms under overfertilization conditions.

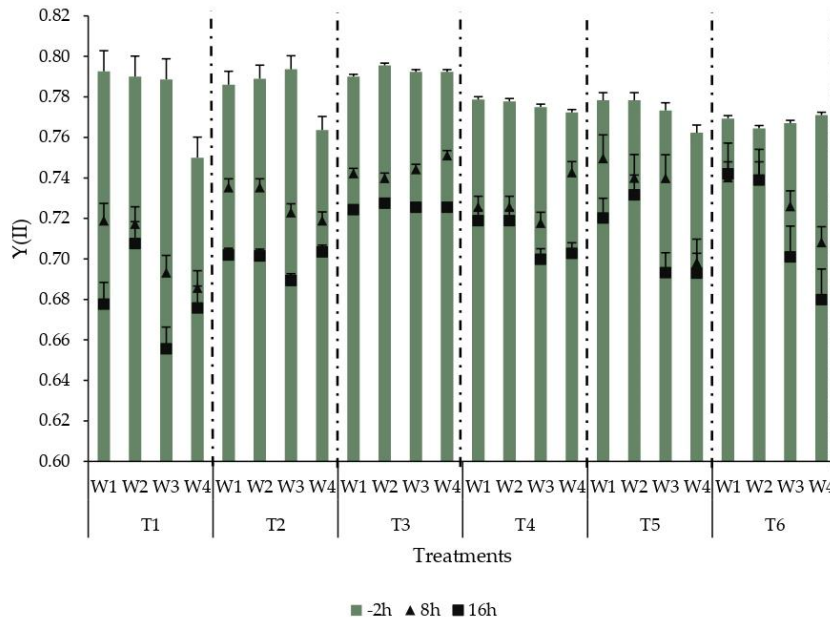


Figure 9. Fv/Fm values in *Lactuca sativa* under different nitrate treatments with *Medicago sativa*. Measurements at -2, 8, and 16 h. Error bars show standard deviation.

Figure 10 shows the non-photochemical dissipation (NPQ) values measured at 8 and 16 hours for four weeks for the different treatments. At 8 hours, variations were observed between treatments and weeks. The 33 % nitrogen treatment (T2) showed the highest value at week 4 (0.568), while the 66 % nitrogen treatment (T3) showed the lowest values, with records below 0.1 in all weeks. At 16 hours, NPQ values increased in most treatments. Treatments 0 % (T1) and 33 % (T2) reached the highest levels, with values up to 0.534 in week 4. In contrast, treatments 66 % (T3) and control (T6) presented lower values, below 0.3 in the same week.

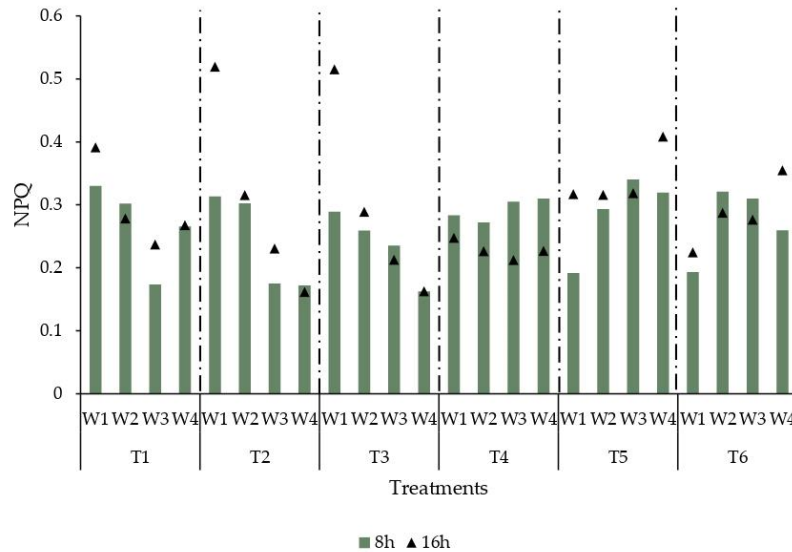


Figure 10. Non-photochemical dissipation (NPQ) at 8 hours (blue bars) and 16 hours (orange dots) during four weeks (W1-W4) in the different treatments.

3.5 Consumo de Energía

Figure 11 shows the results of energy consumption by system component during a production cycle. The highest consumption was recorded in the lighting system, with a total of 293.8 kWh, representing the most significant fraction of total energy use. This value reflects the high energy demand associated with the continuous operation of LED lamps during the extended photoperiod. In second place, the temperature control -which included the operation of the minisplit, fans and exhaust fan- presented a consumption of 206.1 kWh per cycle. The irrigation system, composed of submersible pumps and controllers, registered an intermediate consumption of 99.7 kWh, while the general automation system (Raspberry Pi, sensors, relays and ESP32 modules) showed significantly lower consumption (11.3

kWh). Finally, the CO₂ control had the lowest consumption, with only 2.7 kWh. These results show a clear concentration of energy use in the lighting component, followed by climate control and irrigation, while the auxiliary systems operated with high energy efficiency..

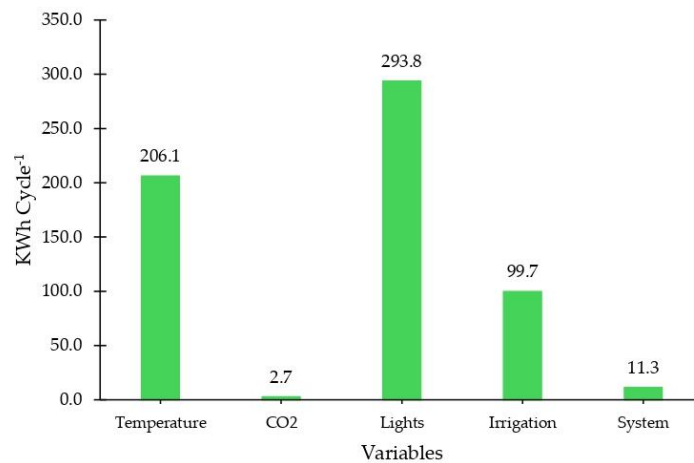


Figure 11. Energy consumption (kWh cycle⁻¹) of the main components of the cultivation system: temperature control, CO₂ enrichment, lighting, irrigation and automation. The data represent the cumulative consumption per variable during a production cycle.

4. Discussion

This study demonstrates that the integration of *Medicago sativa* into controlled NFT systems significantly promotes improvements in growth, photosynthetic efficiency, and resource use in *Lactuca sativa*, particularly under moderate nitrogen supply conditions.

The data obtained reinforce the hypothesis that symbiotic association contributes to ionic stability and physiological performance maintenance, consistent with recent findings in vertical farming systems [2]

The biomechatronic environment developed in this study showed high functionality by maintaining optimal environmental conditions, including a PPFD of $260 \pm 40 \mu\text{mol m}^{-2} \text{s}^{-1}$, with controlled temperature, humidity, and CO_2 levels, aligned with parameters recommended for efficient *Lactuca sativa* production in controlled agriculture systems [21]. The system's ability to sustain these parameters without frequent manual interventions confirms its technological robustness and ensures that the observed physiological responses were exclusively due to the experimental treatments, reinforcing the scientific validity of the model.

The results demonstrate that a moderate nitrogen supply (66% of the standard level), combined with interspecific association with *Medicago sativa*, significantly enhanced photosynthetic and energy efficiency in hydroponic *Lactuca sativa* cultivation. This strategy achieved high quantum efficiency values ($Y(\text{II}) = 0.7467$, $F_v/F_m = 0.7591$) and minimal non-photochemical quenching ($\text{NPQ} < 0.01$), reflecting a functional and stable PSII.

These findings are consistent with those reported by [22], who showed that intermediate nitrogen levels maximize photosynthetic efficiency in hydroponic lettuce, avoiding deficiency or toxicity problems. Similarly, [21] emphasized the importance of a proper balance of nitrogen, light, and CO_2 to maintain high $Y(\text{II})$ and F_v/F_m indices and minimize NPQ under intensive vertical farming conditions.

The symbiotic association with *M. sativa* additionally contributed to improved ionic homeostasis in the rhizosphere, favoring crop physiological stability, as supported by mechanisms described by [23] in controlled systems. In contrast, treatments with

nitrogen deficit (0%, 33%) or excess (100%, 133%) caused marked reductions in Fv/Fm (up to 17.5% under total nitrogen absence) and increases in NPQ above 0.10, evidencing severe physiological stress. This pattern aligns with the observations of [24], who reported that abiotic stress conditions, such as salinity stress, cause decreases in Fv/Fm and increases in NPQ in *Lactuca sativa*, reflecting PSII deterioration and activation of photoprotective energy dissipation mechanisms.

In this study, nitrogen deficit (0%, 33%) and excess (100%, 133%) led to up to a 17.5% reduction in Fv/Fm and NPQ increases greater than 0.10, indicating severe physiological stress. These results align with those of [25], who found that under high light stress, Fv/Fm decreased by over 15%, and NPQ increased by up to 300% as a protective mechanism for PSII. Additionally, [26] highlighted that nitrogen imbalance reduces photosynthetic efficiency, while [27] established that Fv/Fm values below 0.70 are clear indicators of photosynthetic dysfunction under stress.

[28] further explained that NPQ increases reflect the activation of thermal dissipation to protect the photosynthetic apparatus. Altogether, these findings emphasize that balanced nitrogen management and monitoring of photosynthetic parameters (Fv/Fm, Y(II), NPQ) are essential to optimize stability and productivity in intensive hydroponic systems.

SPAD index measurement was confirmed as a reliable tool for estimating chlorophyll and nitrogen content in hydroponically grown *Lactuca sativa* [29]. In this study, treatments with 66% and 100% nitrogen showed high SPAD values (~50–53), levels that, according to the literature, correspond to leaf nitrogen contents above 3.5% dry weight, sufficient to sustain high photosynthetic efficiency and optimal yield [30,31]. Conversely, the 0% nitrogen treatment recorded SPAD values below 40, a physiological threshold indicating severe nitrogen deficiency, associated with

reduced photosynthetic rates and productivity. These results also agree with [32], who found that SPAD values below 45 negatively affect net carbon assimilation and growth in hydroponic lettuce systems. Overall, the data confirm that while symbiotic association with *Medicago sativa* improves nitrogen use efficiency under moderate fertilization, it cannot completely substitute nitrogen fertilization needs.

The 66% nitrogen treatment significantly improved energy performance in *Lactuca sativa*, achieving a LUE of $0.82 \text{ g DW mol}^{-1}$ and an EUE of $1.37 \text{ g FW kWh}^{-1}$, exceeding reference values reported by [33], who found EUEs between 0.95 and $1.35 \text{ g FW kWh}^{-1}$ and LUEs of approximately $0.68 \text{ g DW mol}^{-1}$ for indoor-cultivated lettuce. In contrast, nitrogen excess (133%) reduced LUE and EUE by 16.7% and 14.3%, respectively, evidencing a decrease in physiological efficiency. This behavior matches observations by [34], who reported EUE reduction with increasing Daily Light Integral (DLI), and by [35], who showed that even under optimized lighting, excess irradiance or nutrients diminish photosynthetic efficiency. These findings highlight the importance of balanced nitrogen and environmental management to maximize energy efficiency in controlled agricultural systems.

The total energy consumption during the production cycle was mainly distributed among lighting (293.8 kWh; 46.7%), temperature control (206.1 kWh; 32.8%), irrigation (99.7 kWh; 15.8%), automation system (11.3 kWh; 1.8%), and CO₂ control (2.7 kWh; 0.4%). This distribution confirms that artificial lighting remains the major energy consumer, followed by climate control systems. The high contribution of these two factors emphasizes the need for smarter strategies in photoperiod and thermal control management based on the real physiological response of photosystem II (PSII). This trend is consistent with findings in indoor agricultural systems, where lighting typically accounts for 45–70% of total energy consumption [33].

Unlike studies focusing mainly on monitoring environmental variables such as electrical conductivity (EC), pH, or temperature [36,37], this work incorporated physiological crop evaluation through fluorometry (JUNIOR-PAM) and SPAD index measurements. This strategy enabled a more accurate characterization of PSII functional status in *Lactuca sativa*, complementing classical environmental sensing. Although automatic adjustments based on these physiological data were not implemented, the inclusion of photosynthetic parameters represents progress towards more intelligent agricultural management systems, aligning with recent approaches that advocate physiological feedback as a tool to optimize crop production in controlled environments [38].

This cultivation model has the potential to improve the productivity of urban agricultural systems, reduce fertilizer use by up to 33%, decrease energy consumption per biomass unit, and prevent light stress. The use of PSII sensors allows dynamic adjustments and avoids resource oversizing. Ultimately, the 66% nitrogen treatment combined with symbiosis with *Medicago sativa* proved to be the most efficient in both physiological and energetic terms. The proposed biomechatronic system outperforms traditional configurations and represents a viable strategy for intelligent, sustainable, and quantitatively eco-physiological evidence-based vertical agriculture.

5. Conclusions

Results obtained demonstrate that integrating physiological monitoring of photosystem II (PSII) with automated environmental control in a vertical hydroponic system allows the optimization of photosynthetic efficiency, reduction of light stress, improved resource use, and decreased dependence on synthetic

fertilizers through symbiotic association with *Medicago sativa*. Compared to the nitrogen excess treatment (133%), which reduced photosynthetic efficiency by up to 17%, the 66% nitrogen strategy achieved a superior balance between yield and physiological sustainability.

This study further highlights that moderate nitrogen fertilization combined with symbiotic strategies enhances chlorophyll accumulation, photosynthetic performance (Y(II), Fv/Fm), and energy efficiency (LUE, EUE), while minimizing photoinhibition indicators (NPQ). The proposed biomechatronic platform represents an innovative, eco-physiological evidence-based approach for sustainable vertical farming and urban agriculture.

As a future projection, this research opens multiple strategic lines with high scientific and technological potential: validating the biomechatronic system under real commercial conditions with technical and economic scaling; integrating artificial intelligence algorithms to dynamically adjust lighting, irrigation, and nutrition based on real-time physiological indicators such as Y(II) and NPQ; exploring new symbiotic associations with other nitrogen-fixing species; implementing adaptive spectral lighting synchronized with photosynthetic responses; studying the microbiome–plant–sensor interactions in the rhizosphere; and applying advanced energy-efficiency strategies (such as pulsed lighting or passive cooling) to significantly reduce total electrical consumption, especially in the lighting component (>65%).

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34. Yan, Z.; He, D.; Niu, G.; Zhou, Q.; Qu, Y. Growth, Nutritional Quality, and Energy Use Efficiency of Hydroponic Lettuce as Influenced by Daily Light Integrals Exposed to White versus White Plus Red Light-Emitting Diodes. *HortScience* **2019**, 54, 1737–1744, doi:10.21273/HORTSCI14236-19.

Maestría en Ciencias en Ingeniería Mecatrónica
SYNERGISTIC EFFECTS OF NITRATE UNDER LETTUCE ALFALFA ASSOCIATIONS IN
BIOMECHATRONIC NFT VERTICAL FARMING

35. Chen, X.; Li, Y.; Wang, L.; Guo, W. Red and Blue Wavelengths Affect the Morphology, Energy Use Efficiency and Nutritional Content of Lettuce (*Lactuca Sativa* L.). *Sci Rep* **2021**, *11*, 8374, doi:10.1038/s41598-021-87911-7.
36. Wheeler, R.M. Improving Vertical Farming Efficiency through Dynamic Environmental Control. *Frontiers in Science* **2024**, *2*, doi:10.3389/fsci.2024.1465403.
37. Varghese, A.B.; P, D.M. Enhancing Hydroponic Production: A Review of Plant Growth and Health Monitoring System. In Proceedings of the 2024 Second International Conference on Intelligent Cyber Physical Systems and Internet of Things (ICoICI); IEEE, August 28 2024; pp. 492–498.
38. Kernbach, S. Biofeedback-Based Closed-Loop Phytoactuation in Vertical Farming and Controlled-Environment Agriculture. *Biomimetics* **2024**, *9*, 640, doi:10.3390/biomimetics9100640.

APPENDICE 3

Regional, State and International Awards and Recognitions



OTORGA EL PRESENTE

RECONOCIMIENTO

A

Luis Bryant Díaz Andrade

Por haber obtenido el **2do lugar** en la **Categoría Agrícola**
de la Convocatoria *Talento Joven Ciencia Audiovisual 2024*

DR. LUIS ÁNGEL RODRÍGUEZ DEL BOSQUE
Dirección General del INIFAP

AGUASCALIENTES, 23 – 26 DE OCTUBRE DE 2024

Ciencia para vivir





EDUCACIÓN
SECRETARÍA DE EDUCACIÓN PÚBLICA



TECNOLÓGICO
NACIONAL DE MÉXICO

**EL TECNOLÓGICO NACIONAL DE MÉXICO
A TRAVÉS DEL INSTITUTO TECNOLÓGICO DE AGUASCALIENTES**

OTORGA EL PRESENTE

RECONOCIMIENTO

a:

Luis Bryant Díaz Andrade

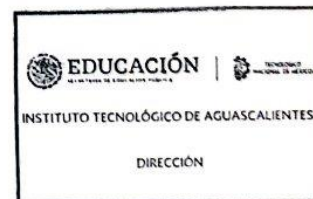
por su participación como:
JURADO CALIFICADOR

de la categoría:
Sector Agroalimentario

durante la **FASE LOCAL** del evento **INNOVATECNM 2024**,
donde estudiantes de la Institución presentaron proyectos de
base tecnológica, y que se llevó cabo el día 09 de mayo de 2024
dentro de las Instalaciones de esta Casa de Estudios.

Aguascalientes, Ags., a 09 de mayo de 2024

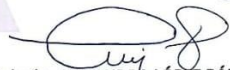
José Luis Gil Vázquez
Director

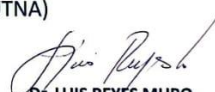




A: Luis Bryant Díaz Andrade y Ruth E. Martínez Escobedo.

Por su participación en Jóvenes en la Ciencia con el tema **“Explorando la agricultura del futuro: Módulos de crecimiento de plantas en entornos biomecatrónicos”** presentado en el III CCCEyS del 29 al 31 de mayo 2024, en modalidad híbrida, con sede en la Universidad Tecnológica del Norte de Aguascalientes (UTNA)


M. en Admón. ALEJANDRA LÓPEZ RÁBAGO
Rectora
Universidad Tecnológica del Norte de
Aguascalientes


Dr. LUIS REYES MURO
Director de Coordinación y Vinculación
del INIFAP en Aguascalientes

Conocimiento Abierto, Rincón de Romo, Ags., Mayo de 2024



Otorga el presente

RECONOCIMIENTO

A:
ING. LUIS BRYANT DIAZ ANDRADE

Por su destacada e invaluable participación
como **CONGRESISTA** en el primer
**Foro Agroalimentario Internacional
Aguascalientes 2023,**
efectuado en la ciudad de Aguascalientes
los días 12, 13 y 14 de septiembre de 2023.



Lic. Isldoro Armendáriz García
Secretario de Desarrollo Rural y Agroempresarial
del Gobierno del Estado Aguascalientes



Lic. Liborio Alberto Flores Mendoza
Presidente Consejo Empresarial Mexicano
de Comercio Exterior, Región Centro Norte



Gustavo Franco Díaz de León
Presidente de la Asociación Ganadera
Local de Aguascalientes

Aguascalientes, Ags., septiembre de 2023



EDUCACIÓN
SECRETARÍA DE EDUCACIÓN PÚBLICA



TECNOLÓGICO
NACIONAL DE MÉXICO

**EL TECNOLÓGICO NACIONAL DE MÉXICO
A TRAVÉS DEL INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA**

OTORGA EL PRESENTE

RECONOCIMIENTO

A

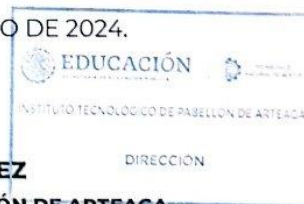
LUIS BRYANT DÍAZ ANDRADE

POR SU DESTACADA PARTICIPACIÓN COMO CONFERENCISTA DENTRO
DEL 8º DÍA DE LAS CIENCIAS BÁSICAS CON EL TEMA "INGENIERÍA VERSÁTIL:
EXPANDIENDO HORIZONTES Y MULTIPLICANDO OPORTUNIDADES" DIRIGIDA
A LAS Y LOS ESTUDIANTES DE ESTA INSTITUCIÓN.

PABELLÓN DE ARTEAGA, A.C.S. A 21 DE MAYO DE 2024.

JOSÉ ERNESTO OLVERA GONZÁLEZ

DIRECTOR DEL INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA





EDUCACIÓN
SECRETARÍA DE EDUCACIÓN PÚBLICA



TECNOLÓGICO
NACIONAL DE MÉXICO

**EL TECNOLÓGICO NACIONAL DE MÉXICO
A TRAVÉS DEL INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA**

OTORGA EL PRESENTE

RECONOCIMIENTO

A

LUIS BRYANT DIAZ ANDRADE

POR HABER IMPARTIDO LA CONFERENCIA
"TECNOLOGÍA LED MULTIESPECTRAL EN UNA GRANJA VERTICAL
BIOMEATRÓNICA PARA LA OPTIMIZACIÓN Y CUANTIFICACIÓN DE
LA FIJACIÓN DE NITRÓGENO EN LECHUGA" DIRIGIDA A LAS Y LOS
ESTUDIANTES DE ESTA INSTITUCIÓN EN EL MARCO DEL
3º SEMINARIO DE MECATRÓNICA.

PABELLÓN DE ARTEAGA, AGS. A 01 DE DICIEMBRE DE 2023.

JOSÉ ERNESTO OLVERA GONZÁLEZ
DIRECTOR DEL INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA





EDUCACIÓN
SECRETARÍA DE EDUCACIÓN PÚBLICA



TECNOLÓGICO
NACIONAL DE MÉXICO

**EL TECNOLÓGICO NACIONAL DE MÉXICO
A TRAVÉS DEL INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA**

OTORGA EL PRESENTE

RECONOCIMIENTO

A

LUIS BRYANT DÍAZ ANDRADE

POR SU DESTACADA PARTICIPACIÓN COMO **EXPOSITOR DE PROYECTOS** Y POR SU INVALUABLE APOYO EN LA PROMOCIÓN DE LA OFERTA EDUCATIVA DE NUESTRO TECNOLÓGICO EN LA **EXPO UNIVERSIDADES 2023** DEL INSTITUTO DE EDUCACIÓN DE AGUASCALIENTES, LOS DÍAS 22 Y 23 DE MARZO

PABELLÓN DE ARTEAGA, AGS. A 23 DE MARZO DE 2023

JOSÉ ERNESTO ÓLVERA GONZÁLEZ
DIRECTOR DEL INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA





EL CENTRO DE INVESTIGACIONES EN ÓPTICA, A.C. (CIO) UNIDAD AGUASCALIENTES
Y EL POSGRADO INTERINSTITUCIONAL EN CIENCIA Y TECNOLOGÍA (PICYT) OTORGAN EL PRESENTE

RECONOCIMIENTO A:

Luis Bryant Díaz Andrade

POR SU VALIOSA PARTICIPACION EN LA 2DA. EDICIÓN DEL SEMINARIO DE DIVULGACIÓN
"LA CATRINA CIENTÍFICA" CELEBRADO DEL 8 AL 10 DE NOVIEMBRE DE 2023

Aguascalientes, Ags. a 10 de Noviembre de 2023

DR. RAFAEL ESPINOSA LUNA
DIRECTOR GENERAL DEL CIO

DR. RICARDO VALENZUELA GONZÁLEZ
COORDINADOR DEL CIO UNIDAD AGUASCALIENTES



GOBIERNO DEL ESTADO
de AGUASCALIENTES

UPA
UNIVERSIDAD POLITÉCNICA
del AGUASCALIENTES

UTNA
UNIVERSIDAD TECNOLÓGICA
del AGUASCALIENTES

UTP
UNIVERSIDAD TECNOLÓGICA
de PUEBLA



AGRICULTURA
SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL

inifap
Instituto Nacional de Investigaciones
Forestales, Agrícolas y Pecuarias



III CICLO DE CONFERENCIAS EN
CLIMA, ENERGÍA Y SUSTENTABILIDAD 2024

CONSTANCIA

LUIS BRYANT DIAZ ANDRADE

Por su participación como **ASISTENTE** en el III CCCEyS del **29 al 31 de mayo 2024**, en
modalidad híbrida con duración de **18 h**, con sede en la Universidad Tecnológica del
Norte de Aguascalientes (UTNA)

Dra. Lidia Elizabeth Vazquez Grajeda
Comité Organizador
Universidad Tecnológica del Norte de
Aguascalientes

Dr. Arturo Corrales Suastegui
Comité Organizador
Instituto Nacional de Investigaciones
Forestales, Agrícolas y Pecuarias-CEPAB

Conocimiento Abierto, Rincón de Romos, Ags., Mayo de 2024

Courses and Trainings



Número de certificado: UC-6b2e29ec-a5f7-4e8f-abd9-0fbb4271f64
Uri del certificado: ude.my/UC-6b2e29ec-a5f7-4e8f-abd9-0fbb4271f64
Número de referencia: 0004

CERTIFICADO DE FINALIZACIÓN

Curso Maestro de Python: De Cero a Programador Todoterreno

Instructores **Héctor Costa Guzmán**

Luis Bryant Diaz Andrade

Fecha **25 de marzo de 2025**
Duración **36.5 horas en total**



CERTIFICADO
FOLIO : D61CAC19 -1E9C-40DC-A567-472A6ACCCFFD

PARA ACREDITAR QUE
LUIS BRYANT DIAZ ANDRADE

COMPLETÓ Y APROBÓ EL CURSO
Electricista

PRESENTANDO A LA FECHA UN AVANCE DE 100% Y UN PUNTAJE DE 9.29.

FECHA Y HORA DE INSCRIPCIÓN : 11 ENE. 2023 - 09:23 A. M.
FECHA Y HORA DE TÉRMINO : 11 ENE. 2023 - 12:00 A. M.

EQUIVALENTE A 46 HORAS AL 100%.

 **Capacitate**
para el empleo

 **FUNDACIÓN**
Carlos Slim

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<https://capacitateparaempleo.org/verifica/baa12c52-71fe-4fd7-8952-b0413535ee0d01cac19-1e9c-40dc-a567-472a6accffD>



CERTIFICADO

OTORGADO A

*LUIS BRYANT DIAZ
ANDRADE*

Introducción al arbitraje y los diferentes modelos de
revisión por pares - TecNM

13 mar. 2024

Fecha de finalización

IOP Publishing

Organizador

IOP
latinoamérica

CERTIFICADO

FOLIO: 36B61F8F-7CBA-4A72-A52F-6B2431E5D1E5

PARA ACREDITAR QUE

LUIS BRYANT DIAZ ANDRADE

COMPLETÓ Y APROBÓ EL CURSO

Técnico en cultivos vegetales

PRESENTANDO A LA FECHA UN AVANCE DE 100% Y UN PUNTAJE DE 9.32.

FECHA Y HORA DE INSCRIPCIÓN : 24 NOV. 2022 - 01:26 P. M.

FECHA Y HORA DE TÉRMINO : 05 ENE. 2023 - 12:00 A. M.

EQUIVALENTE A 62 HORAS AL 100%.



FUNDACIÓN
Carlos Slim

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Human Resource Formation



Instituto Tecnológico de Pabellón De Arteaga

Evaluación Por Docentes



Tipo De Evaluación: Por alumnos

Periodo: AGO-DIC/2023

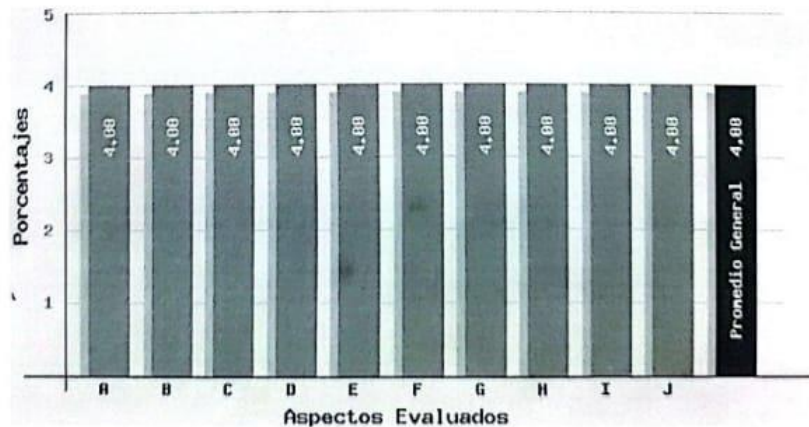
Evaluación Por Docente: DIAZ ANDRADE LUIS BRYANT

Materias			Alumnos	
Clave	Grupo	Nombre Completo De La Materia	Inscritos	Evaluaron
INJ1011	MA4	ESTUDIO DEL TRABAJO I	24	17
TOTAL:			24	17

Aspectos a Evaluar:

Aspectos Evaluados	Puntaje	Calificación
A) Dominio de la asignatura	5	Excelente
B) Planificación del curso	5	Excelente
C) Ambientes de Aprendizaje	5	Excelente
D) Estrategias, metodos y tecnicas	5	Excelente
E) Motivacion	4	Muy Bien
F) Evaluacion	5	Excelente
G) Comunicacion	5	Excelente
H) Gestion del curso	5	Excelente
I) Tecnologias de la Informacion y Comunicacion	5	Excelente
J) Satisfaccion general	5	Excelente
TOTAL:	4.9	Muy Bien

Resultado Grafico:



Maestría en Ciencias en Ingeniería Mecatrónica
SYNERGISTIC EFFECTS OF NITRATE UNDER LETTUCE ALFALFA ASSOCIATIONS IN
BIOMECHATRONIC NFT VERTICAL FARMING

ACTA DE CALIFICACIONES

DEPARTAMENTO: DEPARTAMENTO DE CIENCIAS ECONOMICO ADMINISTRATIVAS
MATERIA: ESTADÍSTICA INFERENCIAL I
PROFESOR: DIAZ ANDRADE LUIS BRYANT
PERIODO: AGO-DIC/2024

FOLIO: 15240226-F
CLAVE: AEF1024
GRUPO: N3A
ALUMNOS: 24

LUNES		MARTES		MIÉRCOLES		JUEVES		VIERNES		SABADO		DOMINGO	
HORA	AULA	HORA	AULA	HORA	AULA	HORA	AULA	HORA	AULA	HORA	AULA	HORA	AULA
7:00 - 8:00	B5	7:00 - 8:00	B5	7:00 - 8:00	B5	7:00 - 8:00	B5	7:00 - 8:00	B5				
No.	No. CONTROL	NOMBRE DEL ALUMNO						CARRERA	REP.	ORD.	COMP.	ESP.	
1	211050302	AGUILAR CAMPOS CARLOS ALONSO						IIND	Si	NA	70		
2	211050307	AGUILAR CAMPOS CARLOS HERANDY						IIND	Si	NA	70		
3	231050201	ALVARADO CAMARILLO KAROL JOCELYN						IIND		83			
4	231050200	CALZADA CAMPOS CRISTOPHER						IIND		93			
5	231050219	CERVANTES JAIME CARLOS DANIEL						IIND		92			
6	231050227	CRUZ GALLEGOS PAULINA JAZMIN						IIND		95			
7	211050313	DIAZ CONTRERAS JOEL DANIEL						IIND		NA	82		
8	231050206	ESCOBEDO LOPEZ DANIEL MAXIMILIANO						IIND		NA	79		
9	231050203	ESPARZA ESPARZA SERGIO JESUS						IIND		NA	70		
10	A221050669	GALINDO MATA JESUS ALEJANDRO						IIND	Si	NA	70		
11	231050208	GARCIA MARMOLEJO DAVID MAXIMILIANO						IIND		NA	84		
12	221050215	GUTIERREZ PARGA LEONEL						IIND	Si	NA	72		
13	231050216	HERRERA IBARRA YESSSENIA CAROLINA						IIND		NA	77		
14	231050212	JIMENEZ GONZALEZ DIEGO IVAN						IIND		93			
15	231050209	LOERA GONZALEZ MARCO ANTONIO						IIND		84			
16	231050218	MURILLO PADILLA JOSE ANDONY						IIND		85			
17	231050229	PIZAÑA VALADEZ MARIJOSE						IIND		NA	81		
18	231050225	PRIETO NUÑEZ JORDAN MATEO						IIND		85			
19	231050221	RAMIREZ HERRERA DAISY ANAHI						IIND		82			
20	231050224	RAMIREZ MENDEZ FATIMA MONSERRAT						IIND		NA	88		
21	221050224	REYES TAVAREZ BRAYAN ANGEL						IIND		NA	80		
22	231050233	REYES SANCHEZ WALDO GUADALUPE						IIND		80			
23	231050232	VALLEJO PUENTES JORGE						IIND		90			
24	231050213	VILLANUEVA RODRIGUEZ FERNANDO						IIND		NA	80		



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Pabellón De Arteaga, Ags., a 16 de Diciembre del 2024

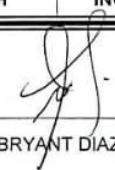
Firma del Profesor:

[Handwritten Signature]

 SECRETARÍA DE EDUCACIÓN PÚBLICA	INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA	
	Acta de Calificaciones de Residencia Profesional	FOLIO: RP-AD24-0050
Empresa o Institución: INSTITUTO TECNOLÓGICO DE PABELLÓN DE ARTEAGA		
Proyecto: MÉTODOS ESTADÍSTICOS AVANZADOS ESTOCÁSTICOS Y NO ESTOCÁSTICOS, PARA LA EVALUACIÓN E INTERPRETACIÓN DE VARIABLES EN LA FIJACIÓN DE NITRÓGENO EN UN ENTORNO BIOMECATRÓNICO DE AGRICULTURA VERTICAL.		
Período de la Residencia: AGOSTO- DICIEMBRE 2024		
Asesor(es) Externo(s): DRA. NIVIA IRACEMI ESCALANTE GARCIA		
Asesor(es) Interno(s): ING. LUIS BRYANT DIAZ ANDRADE		

No.	Núm. Control	Nombre	Carrera	Calificación	Observaciones
1	201050286	JAIME REYES AMERICA JANETH	INGENIERÍA INDUSTRIAL	100	Cien

Escala de calificaciones de 0 a 100, la calificación mínima aprobatoria es 70.
Pabellón de Arteaga, Ags. a 13 de enero de 2025.


ING. LUIS BRYANT DIAZ ANDRADE
NOMBRE Y FIRMA DEL ASESOR INTERNO

