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Chapter 1

COMPARATIVE ANALYSIS OF WATER CULTURE (HYDROPONIC) SYSTEMS IN SOILLESS AGRICULTURE: CURRENT APPROACHES AND FUTURE PERSPECTIVES

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1.INTRODUCTION

The increase in the world's population from 1.6 billion to 7.6 billion inhabitants since the beginning of the 20th century, and the forecast that it will grow to over 10 billion by 2050, as well as a significant increase in per capita agricultural consumption, have been made possible by the expansion and increase of agricultural production, but have also begun to place increasing strain on the agricultural sector (FAO, 2025).

In this context, sustainable and innovative approaches such as Controlled Environment Agriculture (CEA) are gaining importance in increasing the resilience of the food supply chain (Riggio et al., 2019; Chowdhury et al., 2024; Elia et al., 2025).

Operating as the most widely adopted branch of soilless farming, hydroponics fundamentally replaces the traditional soil matrix. Instead, these setups directly supply the rhizosphere with a precisely calibrated liquid solution containing all requisite water, oxygen, and inorganic nutrient ions (Sambo et al., 2019; Resh, 2022). The transition towards industrial-scale implementation of this technology has accelerated rapidly in recent years. This shift is primarily driven by the system's ability to curb evapotranspiration losses-achieving up to 90% water conservation relative to conventional open-field methods-while simultaneously pushing spatial biomass production to its absolute limits, particularly within vertically integrated architectures (Lages Barbosa et al., 2015; Al-Kodmany, 2018; Beacham et al., 2019; Son et al., 2019).

Examining the current literature and industrial applications reveals that hydroponic systems are no longer merely a niche research area but have evolved into large-scale commercial models. Massive indoor vertical farming facilities and modular container farms, established to meet the food demand of metropolises locally, are the most concrete examples of this transformation (Benke and Tomkins, 2017; Petersen et al., 2022). In dense metropolises, the production of special variety premium fruits for the high-end gastronomy market or "baby leaf" greens grown in rapid cycles in highly sensitive systems like RDWC (Recirculating Deep Water Culture) proves the economic feasibility of urban agriculture (Rufí-Salís et al., 2020).

However, despite this high potential offered by hydroponic cultivation, current review studies that comprehensively compare different system designs (DWC, NFT, Aeroponics, Ebb and Flow, etc.) in the context of specific crop groups, resource use efficiencies, and operational risks remain limited in the literatüre (Chowdhury et al., 2024; Guruchandran et al., 2024). The hydrodynamic structure, root zone oxygenation capacity, and energy dependence of each system differ from one another; this situation complicates the selection of the "optimal system" depending on the targeted crop pattern

or the scale of the facility to be established (Lennard and Ward, 2019; Conversa et al., 2021; Yang et al., 2022; Chowdhury et al., 2024).

The aim of this review study is to examine the mechanical working principles of modern hydroponic cultivation methods and to present a detailed comparative analysis of the systems based on plant compatibility, resource efficiency (water and nutrients), operational advantages, and constraints. In this context, the study aims not only to provide a summary of existing systems but also to shed light on the future perspectives of soilless agriculture, such as autonomous management systems and innovative substrate development.

2.CLASSIFICATION AND WORKING PRINCIPLES OF WATER CULTURE (HYDROPONIC) SYSTEMS

The primary objective of hydroponic systems is to maintain the delicate balance between water, oxygen, and nutrient elements in the plant root zone (rhizosphere) at an optimum level (Clyde-Smith and Campos, 2023; Resh, 2022). Hydroponics is basically about managing the root zone. You have to balance water, air, and food in that specific space. The system needs to change this mix as the plant gets older. Generally, we see five main ways to set this up:

1.1. Liquid/Water Culture Systems

Liquid/Water Culture Systems (LWTCs) are a hydroponic farming method where the node is held in a nutrient-rich, oxygenated water solution instead of soil. These systems support healthy, productive, high-yield, soilless farming, ensuring crops have continuous access to nutrients (Zheng et al., 2021).

- **Kratky Method:** This is the easiest way to grow. No power is needed. As the solution gets used up, the water level drops down. This leaves an air pocket. The upper roots use that pocket to breathe. The lower roots stay submerged to eat (Kratky, 2008).

- **Deep Water Culture (DWC):** Deep Water Culture (DWC) is a hydroponic technique that supplies water which contain nutrient direct to the roots of the plant continuously. This technique will ensure the roots of the plant always submerge in water and oxygen. The advantage of DWC system is highly oxygenated uses less fertilizer and low maintenance cost and monitoring time. The rhizosphere, stripped of the physical resistance normally found in soil, absorbs water and inorganic ions almost effortlessly. As a direct result, commercial growers greatly favor this setup for leafy greens, taking advantage of the explosive plant growth rates it reliably provides.

- **Recirculating Deep Water Culture (RDWC):** RDWC takes the core premise of suspending bare roots in water and radically scales the concept by linking isolated growth modules into a continuous, closed-loop aquatic

circuit. Rather than allowing the roots to sit in still liquid, the nutrient broth is forcefully driven from a master command reservoir, flowing through each consecutive bucket before draining back to be re-dosed. This perpetual transit acts as a powerful mechanical equalizer, stabilizing thermal gradients and locking electrical conductivity perfectly in place across the entire growing floor. Facility operators strongly favor this unified architecture simply because balancing the chemistry of one massive, moving body of water is infinitely more reliable and less labor-intensive than micromanaging dozens of disconnected vats. RDWC maintains superior Electrical Conductivity (EC) and pH stability on a large scale.

1.2.Continuous Flow Systems

In these systems, instead of a deep pool, roots are fed by a shallow and continuously flowing layer of solution.

- **Nutrient Film Technique (NFT):** A system where plant roots are placed in narrow channels with a 1-3% slope. While only the lower tips of the roots touch the continuous 1-2 mm thick nutrient flow, the upper parts absorb oxygen from the moist air within the channel (Chowdhury et al., 2024; Lakhiar et al., 2018).

- **Vertical NFT:** Vertical NFT represents a structural evolution of standard film techniques, stacking the continuous-flow channels into hyper-dense, multi-story arrays (Erekath et al., 2024). In the context of massive, climate-controlled farming operations that encompass vast footprints, traditional flat-plane growing simply wastes overhead space. By shifting the paradigm to these gravity-fed configurations, facility engineers can exponentially multiply the raw biomass extracted from every single cubic volume (m^3) of the building. Ultimately, relying on natural gravity to carry the thin nutrient layer down through successive vegetative tiers is the defining design choice that makes mega-scale indoor agriculture economically and spatially viable (Graamans et al., 2018).

1.3.Aeroponic Systems (Air Culture and Fogging)

Aeroponic systems are advanced technology applications where water is atomized and sprayed directly onto bare roots, maximizing oxygenation. Systems are divided into three based on the pressure used and the generated droplet diameter (Lakhiar et al., 2018).

- **Low-Pressure Aeroponics (LPA):** Relying heavily on standard commercial water pumps, LPA configurations essentially fall short of true atomization. The hardware forces out relatively large liquid particles-well over 100 μm in diameter-which physically restricts how effectively the root mass can capture moisture and inorganic ions. Consequently, this structural

bottleneck keeps the plant's overall nutrient uptake rates from ever reaching their absolute peak (Martin-Laurent et al., 2000).

- **High-Pressure Aeroponics (HPA):** Among the multitude of soilless production systems, high-pressure aeroponics (HPA) are relatively recent and have received the greatest amount of interest in the literature. With this technology, the roots are kept bare in a humid atmosphere, and they are regularly sprayed with a very fine mist that is generated from a pressurized nutrient solution. This method is of particular interest when easy access to the roots is needed or when the artificial substrates used for soilless culture are banned to prevent them from impacting the environment through their disposal or production. Across agronomic literature, this highly specific micro-scale is universally recognized as the biological sweet spot. Because the physical dimensions of the mist perfectly match the architecture of delicate capillary roots, the plant is able to assimilate moisture and dissolved ions at absolute peak velocity (Lakhiar et al., 2018; Kumari and Kumar, 2019).

- **Fogponics (Ultrasonic Aeroponics):** Systems where water is converted into a 5-10 micron visible fog via piezoceramic ultrasonic discs. Although oxygen transmission is excellent, systemic blockages due to salt accumulation are a significant constraint (Li et al., 2018; Kumari and Kumar, 2019; Suganob et al., 2024).

1.4.Media-based and Cyclical Systems

Deviating from pure liquid environments, this specific architectural approach secures the plant's developing root mass within an inert physical matrix or a rigorously engineered aggregate blend. By meticulously formulating these structural substrates—often combining components like expanded perlite and peat moss, occasionally stabilized with specialized organic binders-agronomists can exert highly precise, localized control over both the structural anchoring of the crop and the exact moisture retention dynamics of the rhizosphere (Gruda, 2019).

- **Ebb and Flow:** Ebb and Flow systems do not hold water constantly. A pump floods the plant tray with nutrient solution. The pump then turns off. Gravity pulls the liquid back down into a main tank. The drainage part is the most important step. The falling water creates a natural vacuum inside the grow medium. It physically pulls fresh air down to the roots. The plants get a massive supply of oxygen during every single cycle.Consequently, this mechanical breathing process guarantees that the rhizosphere receives aggressive oxygenation between feeding intervals, completely neutralizing the risk of root hypoxia (Son et al., 2019).

- **Drip Systems:** Drip systems do not flood the entire growing area. A network of small emitters targets each individual plant. The roots get precise

drops of the nutrient solution. The grower gets total control over the moisture level. You can even dry the root zone out on purpose. This intentional drought stress forces the crop to produce more secondary metabolites. High-value plants benefit greatly from this exact watering method. A prime example of this is the commercial production of ultra-premium strawberry cultivars-particularly highly sought-after Japanese and Korean genetic lines-destined for top-tier gastronomy markets. In these niche sectors, manipulating the root environment with surgical precision is what ultimately dictates the final sugar concentration and complex flavor profile of the fruit (Resh, 2022).

1.5. Aquaponic Systems

Integrated Bio-Agricultural Cycles Aquaponics is an innovative bio-agricultural approach combining closed-loop aquaculture and soilless agriculture (hydroponics) in a closed and symbiotic ecosystem (Somerville et al., 2014). Instead of external dosing of inorganic chemical fertilizers (pure hydroponics), this system is based on the principle of using organic waste found in fish feces as the primary nutrient source for plants.

The heart and driving force of the system, alongside mechanical filtration, are the nitrifying bacteria colonizing the biofilter unit. Ammonia (NH_3/NH_4^+) toxicity is a massive issue in aquaculture. Fish gills release it constantly. You need a two-step bacterial process to fix this before watering any plants. Nitrosomonas bacteria do the first part. They turn the ammonia into NO_2 , which is nitrite. Then Nitrobacter or Nitrospira strains finish the sequence. They convert the nitrite into NO_3^-, or nitrate (Goddek et al., 2019; Goddek et al., 2020). Plants grow very well in DWC or NFT beds using this exact nitrate. The extensive root systems pull the oxidized nitrogen right out of the water. This massive root uptake naturally filters the effluent. The whole process purifies the liquid. The clean water safely returns to the fish basin (Figure 1).Compared to the meticulous chemical control of pure hydroponic systems, aquaponics is a “compromise” management requiring the simultaneous provision of optimum living conditions for three different biological actors (fish, plants, and bacteria) (Yep and Zheng, 2019).

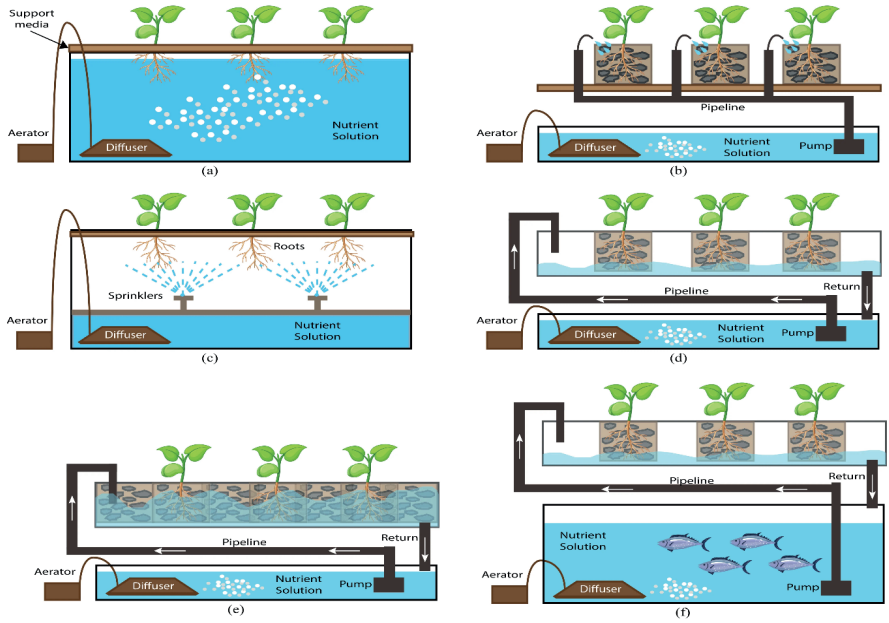


Figure 1. *Different types of hydroponic systems. (a) Deep Water Culture. (b) Drip System. (c) Aeroponics. (d) Nutrient Film Technique (NFT). (e) Ebb and flow. (f) Aquaponics (Velazquez-Gonzalez et al., 2022)*

2. COMPARATIVE ANALYSIS (CRITICAL ASSESSMENT)

The performance of different hydroponic systems varies greatly depending on the targeted crop pattern, the climate control capacity of the facility, and the targeted economic scale. Therefore, rather than an "ideal" hierarchy of systems, a comparative analysis based on specific parameters is required (Kozai and Niu, 2016).

2.1. Resource Use Efficiency: Water and Nutrient Dynamics

Water Use Efficiency (WUE), one of the primary motivations for Controlled Environment Agriculture (CEA), shows significant differences among systems. Although all hydroponic systems provide water savings compared to traditional agriculture, aeroponic systems offer the highest efficiency with a WUE value in the 95-98% range. In NFT and DWC systems, this ratio is around 80-90%. While the atomization of water in aeroponic systems minimizes evaporation losses, evapotranspiration losses are relatively higher in stagnant water systems with a large surface area like DWC.

In terms of Nutrient Use Efficiency (NUE), recirculating NFT and RDWC systems offer high efficiency by recovering the leached solution (Rouphael et al., 2018). However, this recirculation process can lead to the accumulation of root exudates in the system and potential phytotoxicity. Conversely, in

run-to-waste drip systems, the risk of nutrient accumulation is near zero, but fertilizer costs increase.

2.2. Crop Suitability and Root Zone Stress Management

Not every hydroponic system provides an optimal cost-benefit ratio for every plant physiology. The compatibility between the hydrodynamics of the system and the growth rate and root structure of the plant is critical (Sambo et al., 2019).

- **Fast-Cycling Leafy Greens:** Plants in the "baby leaf" segment, such as mizuna, arugula, and basil, are highly sensitive to pH and EC fluctuations. RDWC systems allow these species to be harvested in a very short time (20-30 days) due to the flawless chemical stability provided by high water volumes (Son et al., 2023).

- **Value-Added Fruiting Crops:** The indoor cultivation of Korean and Japanese origin strawberry varieties with high brix (sugar) levels and specific aroma profiles requires meticulous management of moisture/drought stress in the root zone. For such premium crops, precision drip systems supported by perlite-peat-based special substrates or high-pressure aeroponic systems yield optimum results rather than RDWC or NFT (Zheng et al., 2021).

- **Sprout and Microgreen Production:** For hyper-fast-cycling crops produced in lightless or low-light, climate-controlled rooms like mung bean sprouts, modified tray systems operating on a drench and drain principle without substrate are superior to DWC and NFT in terms of minimizing disease risk (Singh, 2023).

2.3. Operational Risks and System Buffering Capacity

In commercial hydroponic enterprises, failure tolerance is a vital factor in system selection. Buffering capacity refers to the duration roots can withstand drying and temperature shocks in the event of a power or pump failure. DWC and RDWC systems offer high thermal and biological buffering as they hold tons of liters of water; even if oxygen pumps stop, plants can survive for hours, sometimes days (Karnoutsos et al., 2025).

In contrast, the buffering capacity in NFT and aeroponic systems is near zero. In the event of an interruption in nutrient flow or misting, fine capillary roots dry out within minutes, undergoing irreversible necrosis (tissue death) (Eldridge et al., 2020). Therefore, these systems mandatorily require costly automation and backup generator infrastructures.

2.4. Scalability and Space Optimization

Translating data obtained in a laboratory environment to a commercial scale brings significant engineering constraints. The architectural dynamics of container-type spaces and massive industrial indoor farming facilities of

2,500 square meters and above differ from one another (Benke and Tomkins, 2017).

If the overarching goal is to scale cultivation upward, both aeroponic networks and thin-film techniques (NFT) present a distinct architectural advantage; their inherently negligible fluid mass allows facility engineers to effortlessly stack them into towering, multi-tier configurations. Conversely, attempting to integrate Deep Water Culture into these vertical arrays introduces a severe structural bottleneck. Because DWC reservoirs fundamentally rely on massive volumes of standing liquid-frequently imposing hundreds of kilograms of dead static weight for every square meter of floor space-the heavy-duty architectural reinforcements required to safely support such extreme loads can render the construction of multi-story DWC facilities financially prohibitive (Kozai and Niu, 2020).

Hence, giant indoor vertical farming facilities generally prefer vertical NFT, tower aeroponics, or drip systems with lightweight media (e.g., latex-bound peat/perlite mixtures) to maximize space.

3. CURRENT APPROACHES AND INNOVATIVE APPLICATIONS

Hydroponic systems today have gone beyond being a mere agricultural production method; they have transformed into multidisciplinary industrial processes where bioengineering, materials science, and data analytics intersect. Current studies in the literature indicate that innovative approaches are deepening along four main axes:

3.1.Scaling and Aerodynamic Optimization in Facility Engineering

In Controlled Environment Agriculture (CEA), facility architecture faces critical engineering barriers as it evolves from modular systems to massive industrial structures.

- **Modular and Hyper-Compact Systems:** Autonomous structures created by converting shipping containers offer flexibility for local supply chains. In these systems, biomass per m^3 is maximized using vertical NFT or high-pressure aeroponic infrastructures while ensuring complete isolation from the external climate (Benke and Tomkins, 2017).

- **Industrial Scale Vertical Facilities (Mega-Farms):** In multi-story facilities with an installation area exceeding 2,500 square meters, the primary problem is not water delivery but microclimate management. Heat islands and Vapor Pressure Deficit (VPD) imbalances occurring between layers lead to the cessation of transpiration and calcium deficiency (tipburn) in plants. Current research employs Computational Fluid Dynamics (CFD) modeling to ensure homogeneous airflow in these massive facilities and integrates HVAC systems based on these simulations (Despommier, 2020).

3.2. Crop Steering and Value-Added Crop Adaptations

While the focus of traditional hydroponic production was "rapid growth," current approaches focus on enhancing the secondary metabolites (aroma, sugar, antioxidants) of plants by manipulating climate and nutrient stress (generative steering).

- **Stress Management in Premium Fruits:** Asian-origin strawberry varieties with high Brix (sugar) and specific aroma profiles, which are at the pinnacle of the gastronomy sector, respond poorly to standard irrigation regimes. In current applications, planned "dry-backs" are created in the root zone using drip irrigation systems. By restricting water and momentarily raising the EC (Electrical Conductivity), the plant is forced into a generative phase, dramatically increasing fruit quality.

- **Baby Leaf and RDWC Integration:** The rapid-cycle production of leafy greens such as mizuna, mustard greens, and basil can create physiological problems like nitrate accumulation. In modern RDWC systems, new cultivation recipes are being developed where the nutrient solution is replaced with ultra-pure water 48 hours before harvest, and specific LED spectrums (High Red:Blue ratio) are used to increase ascorbic acid (Vitamin C) levels and reduce nitrate in the plant (Son et al., 2023).

- **Climate-Controlled Full-Darkness Sprouting:** For crops not requiring photomorphogenesis like mung bean sprouts, cyclic drench systems are used in completely isolated rooms where humidity is kept above 90%. The current research focus in these hyper-fast systems is ozone (O_3) generators and UV-C disinfection modules integrated into the solution to prevent bacterial rot.

3.3. Physicochemical Dynamics of Biodegradable and Hybrid Growing Media (Substrates)

To reduce the environmental footprint of soilless agriculture, traditional rockwool is being replaced by hybrid substrates whose physicochemical properties are engineered. Recent breakthroughs in substrate formulation have largely pivoted toward cohesive, non-degrading plug structures rather than loose media. Loose peat and perlite mixtures have a major physical flaw. They shrink. As the roots grow, the whole substrate compacts downward. This ruins the original Air-Filled Porosity (AFP) and Water Holding Capacity (WHC). You lose the exact moisture balance. Substrate companies now use natural latex to glue everything together. The loose fractions get mixed with the liquid binder and then baked. The heat locks the particles in place. You get a solid plug. The roots never run out of oxygen late in the season (Gruda, 2019). The latex binder prevents the macro-pores within the media from collapsing (compaction) over time, averting root suffocation during long-term

production cycles (especially in perennial plants like premium strawberries) and stabilizing the cation exchange capacity (CEC).

3.4. Digital Agriculture: Artificial Intelligence (AI) Algorithms and Computer Vision

Most commercial farms already use sensors to track the environment. This is now a standard practice. However, the focus is shifting toward algorithmic control. Data is no longer just for keeping records. Modern systems put machine learning models directly into the control loop. These models process massive amounts of sensor data instantly. The system then makes its own environmental changes. This removes the need for a person to watch the numbers constantly (Mahmud et al., 2021).

· **Algorithmic Forecasting:** Automation software connects directly to environmental sensors. The system tracks pH, EC, DO, and VPD every single second. Advanced neural networks process all this incoming numbers. The AI catches invisible physicochemical deviations. It fixes the environment without human help. You stop the negative impacts before they hurt the plant metabolism.

· **Optical Diagnostics and Computer Vision:** Standard visual inspections are useless for early warning signs. Multispectral cameras need in the growing area. The optical sensors perceive the light bouncing off the canopy. This specific reflectance signature reveals invisible stress factors. The computer vision spots the abnormality. You intervene before irreversible tissue damage happens.

5. FUTURE PERSPECTIVES AND UNRESOLVED RESEARCH AREAS

While academic literature frequently romanticizes the theoretical efficiency of controlled-environment agriculture, scaling these frameworks to massive commercial grids consistently exposes severe vulnerabilities across their metabolic, mechanical, and financial parameters. We have effectively exhausted the utility of isolated hardware upgrades. The survival and actual scalability of soilless cultivation now dictate a radical, aggressive integration of entirely disparate scientific domains. If contemporary agronomy genuinely intends to dismantle the glaring empirical deficits plaguing current models, subsequent research must rapidly converge on the following interdisciplinary frontiers:

We have effectively exhausted the utility of isolated hardware upgrades. The survival and actual scalability of soilless cultivation now dictate a radical, aggressive integration of entirely disparate scientific domains. If contemporary agronomy genuinely intends to dismantle the glaring empirical deficits plaguing current models, subsequent research must rapidly converge on the following interdisciplinary frontiers:

5.1. Non-linear Scaling Challenges in Mega-Scale Facilities

Extrapolating the architecture of compact, modular growing units-such as retrofitted shipping containers-into hyper-scale industrial operations presents a monumental structural bottleneck for tomorrow's agricultural engineers. Small hydroponic setups do not scale up easily. You cannot just build a massive Nutrient Film Technique or aeroponic system using pilot blueprints. The physics change completely in a large facility. A multi-hectare Plant Factory with Artificial Lighting (PFAL) traps serious metabolic heat. The vapor pressure gradients shift dramatically across huge rooms. Water flow behaves very differently over long distances. Engineers must design these massive grids entirely from scratch. Copying a small model and making it bigger causes immediate biological failures. The entire architecture requires custom calculations for that exact building size (Kozai and Niu, 2020). In large-volume facilities, the aerodynamic friction between rack systems and the boundary layer resistance created by the plant canopy disrupt the homogeneity of the microclimate (temperature, humidity, VPD). Future research should focus on optimizing localized airflow distribution systems and micro-climate control integrated into the plant root and canopy zones, rather than acclimatizing the entire room (macro-climate HVAC).

5.2. Physicochemical Degradation Dynamics and Standardization of Hybrid Substrates

Driven by mounting ecological pressures, the hydroponic sector is currently experiencing a massive material transition, progressively abandoning sterile stone wool in favor of ecologically degradable, hybrid matrices. Despite this rapid industrial adoption, the scientific community has yet to comprehensively profile the prolonged chemical endurance of these novel substrates. For instance, binding loose perlite and peat moss into cohesive blocks via organic polymers introduces complex degradation variables; exactly how these biological adhesives interact with the nutrient solution's chemistry over an extended cultivation cycle represents a glaring gap in current agronomic research (Barrett et al., 2016). Future studies must measure the mechanical degradation rates experienced by these latex-based hybrid media, especially during months-long production cycles (when exposed to acids in the solution and root exudates). There is an urgent need for longitudinal studies to model the risk of fine particles formed by the dissolution of binders clogging drippers or aeroponic nozzles, as well as the loss of Water Holding Capacity (WHC) and Air-Filled Porosity (AFP) of the media over time.

5.3. Development of Specific Cultivation Recipes and Genotype-System Interactions

For decades, the bulk of hydroponic literature has been disproportionately saturated with studies on standard commodity crops, leaving a profound gap in advanced botanical applications. Moving forward, the ultimate trajectory of indoor agronomy hinges on engineering exact "Precision Cultivation Recipes." By developing these meticulously tuned environmental matrices, researchers will finally empower commercial facilities to transition beyond basic greens, securing the successful adaptation of highly lucrative and delicate species within bespoke aquatic frameworks (Son et al., 2023).

- **Premium Strawberry Production:** The premium culinary sector in major urban centers increasingly demands specialized commodities, such as high-Brix Asian strawberry varieties, which absolutely cannot reach their genetic zenith under standard, constant-feed hydroponic protocols. While static nutrient solutions perfectly suit vegetative crops like lettuce, cultivating top-tier strawberries requires complex physiological manipulation. Moving forward, the scientific community must prioritize the development of dynamic fertigation and light-steering recipes. By intentionally fluctuating nutrient concentrations to induce mild abiotic stress—ideally managed within advanced drip or aeroponic frameworks—growers can dramatically amplify the aromatic and flavor profiles of these highly lucrative fruits (Savvas and Gruda, 2018).

- **Hyper-Fast Cycling Crops:** EC buffering dynamics in baby leaf production like mizuna in RDWC systems, and the autonomization of pathogen suppression (hygiene/sanitation) systems in the production of mung bean sprouts grown in completely climate-controlled, isolated dark rooms, are critical niche areas waiting to be explored in the urban agriculture literature.

5.4. Circular Economy and Life Cycle Assessment (LCA)

Whether hydroponic systems are as "green" as claimed, given their high energy consumption (artificial lighting and HVAC), is intensely debated in academic circles. Future studies must transparently analyze not only the biomass yield of different system types (DWC, NFT, Aeroponics) but also their carbon footprint per product and energy cost (kWh/kg) through comprehensive Life Cycle Assessment (LCA) models (Chowdhury et al., 2024).

6. CONCLUSION

In the context of Controlled Environment Agriculture (CEA), water culture (hydroponic) systems offer one of the most viable engineering solutions against resource scarcity and climate uncertainty crises facing modern food production. The comparative analysis conducted within the scope of this

review revealed that systems such as DWC, NFT, Aeroponics, and Ebb and Flow do not possess a universal hierarchy of superiority; instead, each system yields optimum results when paired with specific facility scales, operational capacities, and targeted crop physiologies.

While aeroponic systems provide an undisputed advantage in water and nutrient use efficiency, their high installation costs and operational fragilities limit their commercial adaptation. On the other hand, DWC and RDWC systems offer a reliable infrastructure, especially for fast-cycling leafy crops, with the thermal and chemical buffering capacity provided by their high water volumes. In the stress-based cultivation of premium fruiting plants, precision drip and modified vertical systems supported by hybrid substrates stand out.

In conclusion, the next evolution of soilless agriculture will occur not through radical changes in mechanical system designs, but rather; through the integration of micro-climate engineering, biodegradable substrate optimizations, and autonomous decision support systems (AI/IoT) capable of instantly sensing and managing plant physiology. Handling system components with a holistic approach is the fundamental key to transforming the laboratory-scale successes of hydroponic agriculture into sustainable food supply chains for massive metropolises.

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Chapter 2

SUSTAINABLE SUPERCRITICAL CO_2 EXTRACTION UNLOCKS THE ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF *ISATIS TINCTORIA* SUBSP. *TOMENTELLA*

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INTRODUCTION

Natural products have long been recognized as a valuable source of biologically active compounds with potential applications in medicine, food preservation, and pharmaceuticals. In recent years, increasing concerns regarding oxidative stress and antimicrobial resistance have accelerated the search for plant-derived antioxidants and antibacterial agents (Caliskan et al., 2023; Srivastava et al., 2023).

Isatis tinctoria L., a member of the Brassicaceae family, is widely distributed across Europe, Asia, and the Mediterranean region, including various parts of Türkiye. The plant has been traditionally utilized for its medicinal properties and is known to contain a diverse range of secondary metabolites such as flavonoids, phenolic acids, alkaloids, and glucosinolates. These compounds are associated with multiple biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Güner et al., 2001; Miceli et al., 2023; Speranza et al., 2020).

The efficiency of extracting bioactive compounds from plant matrices is highly dependent on the extraction technique employed. Conventional extraction methods often involve high temperatures and organic solvents, which may lead to degradation of thermolabile compounds and environmental concerns. In contrast, supercritical CO_2 extraction has emerged as an environmentally friendly alternative due to its low toxicity, selectivity, and ability to preserve sensitive compounds under mild conditions (Dupré et al., 2025; Miceli et al., 2023; Speranza et al., 2020; Caliskan et al., 2023; Nicosia et al., 2024; Keskinkaya et al., 2025; Yıldırım et al., 2024).

Although several studies have investigated the biological properties of *I. tinctoria*, limited information is available regarding the bioactivity of extracts obtained specifically through supercritical CO_2 extraction. Therefore, the present study aims to evaluate the antioxidant and antibacterial potential of *I. tinctoria* subsp. *tomentella* extract obtained using this method.

MATERIALS AND METHODS

Plant Material and Extraction

The aerial parts of *I. tinctoria* subsp. *tomentella* were collected from natural habitats in Türkiye. After collection, the plant material was air-dried under shade conditions and subsequently ground into a fine powder.

Extraction was performed using a supercritical CO_2 system under optimized conditions. Carbon dioxide was used as the primary extraction solvent, and ethanol was added as a co-solvent to improve the extraction efficiency of polar compounds. The obtained extract was collected, concentrated, and stored at appropriate conditions until further analysis (Keskinkaya et al., 2025; Akdag et al., 2025).

Determination of Antioxidant Activity

The antioxidant capacity of the extract was evaluated using DPPH• and ABTS•$^{+}$ radical scavenging assays, CUPRAC reducing power assay, and metal chelating activity test. Different concentrations of the extract were prepared, and all measurements were carried out in triplicate. Standard antioxidants were used for comparison (Decker & Welch, 1990; Deveci et al., 2019; Blois, 1958; Keskinkaya et al., 2026).

Determination of Total Phenolic and Flavonoid Contents

Total phenolic content was determined using the Folin–Ciocalteu method, and the results were expressed as gallic acid equivalents. Total flavonoid content was measured using a colorimetric method based on aluminum complex formation and expressed as quercetin equivalents (Slinkard & Singleton, 1977; Park et al., 2004).

Determination of Antibacterial Activity

The antibacterial activity of the extract was assessed using the broth microdilution method. Minimum inhibitory concentration (MIC) values were determined against selected Gram-positive and Gram-negative bacterial strains. Gentamicin was used as a reference antibiotic. (David et al., 2021; Akdag et al., 2025; Basri & Fan, 2005; Keskinkaya et al., 2020; Lourens et al., 2004).

RESULTS

The SIT extract demonstrated notable antioxidant activity across all applied assays. In both DPPH• and ABTS•$^{+}$ methods, the extract exhibited strong radical scavenging capacity, reflected by relatively low IC_{50} values. Although standard antioxidants showed slightly higher activity in terms of percentage inhibition, the extract displayed competitive IC_{50} values, indicating effective radical neutralization potential (Table 1).

In the CUPRAC assay, the extract showed a considerable reducing ability, confirming its electron-donating capacity. On the other hand, its metal chelating activity was lower compared to EDTA, suggesting that its antioxidant effect is primarily associated with radical scavenging and reduction mechanisms rather than metal ion binding (Table 1).

The phytochemical analysis revealed that the extract contained substantial amounts of phenolic and flavonoid compounds. These results are consistent with the observed antioxidant activity, as such compounds are known to contribute significantly to free radical neutralization (Table 2).

Regarding antibacterial activity, the extract exhibited varying levels of inhibition depending on the tested microorganism. The strongest effects were observed against *E. coli* and *K. pneumoniae*, while moderate activity was detected against *S. enteritidis* and *B. cereus*. Lower activity was observed for *S.*

aureus and *S. lutea*, and no inhibitory effect was detected against *P. aeruginosa* (Table 3).

Table 1. *Antioxidant capacity of I. tinctoria subsp. tomentella extracts*

		Antioxidant Activity							
		DPPH• assay		ABTS•+ assay		CUPRAC assay		Metal Chelating assay	
		Inhibition (%)[a]	IC_{50} (µg /mL)[b]	Inhibition (%)[a]	IC_{50} (µg/mL)[b]	Absorbance[c]	$A_{0.50}$ (µg/mL)[d]	Inhibition (%)[a]	IC_{50} (µg /mL)[b]
Extracts	**SIT**	78.65 ± 0.21	**12.40 ± 0.18**	80.12 ± 0.26	**9.80 ± 0.24**	3.18 ± 0.04	**15.60 ± 0.22**	62.35 ± 0.48	**45.30 ± 0.35**
Standards	**BHT**	90.12 ± 0.16	18.10 ± 0.30	86.20 ± 0.28	11.40 ± 0.42	3.55 ± 0.04	21.90 ± 0.22	-	-
	BHA	92.35 ± 0.14	17.60 ± 0.25	88.15 ± 0.30	10.90 ± 0.38	3.72 ± 0.03	20.80 ± 0.20	-	-
	EDTA							95.80 ± 0.35	2.90 ± 0.05

SIT: supercritical CO_2 extract from *I. tinctoria* subsp. *tomentella,* BHT: Butylated hydroxytoluene, BHA: Butylated hydroxyanisole, EDTA: E*thylene-diaminetetraacetic acid*

a: % inhibition of extracts at a concentration of 400 µg/mL.

b: IC_{50} values are given as the mean ±SD of three parallel measurements.

c: Absorbance of extracts at a concentration of 400 µg/mL.

d: $A_{0.50}$ values are given as the mean ±SD of three parallel measurements.

Table 2. *TPC and TFC contents of I. tinctoria subsp. tomentella extract*

Extract	**TPC (µg GAEs/mg extract[b])**	**TFC (µg QEs/mg extract[c])**
***I. tinctoria* subsp. *tomentella*[a]**	318.475 ± 118.6	452.18 ± 24.3

a: The results are given as the mean ± SD of three parallel measurements.

b: GAEs, gallic acid equivalent, y=0.0123x-0.0155 r2=0.9931

c: QEs, quercetin equivalent, y=0.0156x-0.0112 r^2=0.9985

Table 3. *Antibacterial activity (MIC values) of the supercritical CO_2 extract of I. tinctoria subsp. tomentella*

Test Microorganism	MIC (mg/ml)		
	SIT	**Gentamycin (0.1 mg/ml)**	**DMSO**
Escherichia coli	**1.562**	<0.02	12.5%
Pseudomonas aeruginosa	NA*	<0.02	12.5%
Klebsiella pneumoniae	1.562	0.78	12.5%
Staphylococcus aureus	6.25	<0.02	25%
Salmonella enteritidis	3.125	0.04	12.5%
Sarcina lutea	6.25	<0.02	12.5%
Bacillus cereus	3.125	<0.02	12.5%

SIT: I. tinctoria subsp. tomentella supercritical CO_2 extract

**NA: not active*

DISCUSSION

The results obtained in this study indicate that the supercritical CO_2 extract of *I. tinctoria* subsp. *tomentella* possesses biologically relevant antioxidant and antibacterial properties. These activities can be largely attributed to the presence of phenolic and flavonoid compounds identified in the extract.

The strong performance observed in radical scavenging assays suggests that the extract is effective in neutralizing reactive oxygen species. This is particularly important, as oxidative stress is closely associated with the development of various chronic diseases. The relatively high reducing capacity further supports the antioxidant potential of the extract (Caliskan et al., 2023).

The comparatively weaker metal chelating activity indicates that different antioxidant mechanisms contribute unequally to the overall activity profile of the extract. Such variability is common in plant-derived extracts and depends on their chemical composition.(Dupré et al., 2025; Nicosia et al., 2024; Dupré et al., 2025; Miceli et al., 2023).

The antibacterial findings revealed that the extract is more effective against certain Gram-negative bacteria, which may be related to differences in cell wall structure and permeability. The absence of activity against *P. aeruginosa* is not unexpected, as this organism is known for its intrinsic resistance to many antimicrobial agents (Speranza et al., 2020).

In addition, the use of supercritical CO_2 extraction likely played a significant role in preserving bioactive compounds and enhancing extract quality. This method offers advantages over conventional techniques, including reduced solvent residues and improved selectivity (Yıldırım et al., 2024).

CONCLUSION

In conclusion, the present study demonstrates that *I. tinctoria* subsp. *tomentella* extract obtained via supercritical CO_2 extraction exhibits significant antioxidant activity along with moderate antibacterial effects. The biological activities observed are closely associated with the phenolic and flavonoid content of the extract.

These findings suggest that the extract may serve as a promising natural source of bioactive compounds for potential applications in pharmaceutical and food-related fields. Furthermore, the use of an environmentally friendly extraction method enhances the overall value of the study. Future research focusing on compound isolation and in vivo evaluation would provide further insight into its therapeutic potential.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

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Chapter 3

THE ROLE OF MITOCHONDRIA IN DISEASE PATHOGENESIS AND MITOCHONDRIAL TARGETED THERAPEUTIC STRATEGIES

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1. Mitochondria

Mitochondria are double-membraned, intracellular organelles found in all eukaryotic cells that play a fundamental role in cellular energy production (Schlieben & Prokisch, 2023). These organelles contain approximately 16.6 kilobases of circular mitochondrial DNA (mtDNA) and possess their own genome. The genome under consideration encodes the 13 core protein subunits of complexes I, III, IV, and V of the mitochondrial oxidative phosphorylation (OXPHOS) system's respiratory chain, the 22 tRNAs involved in mitochondrial protein synthesis, and the 13 core protein subunits of the mitochondrial subunits required for translation. The fourth and fifth elements of the respiratory chain of the mitochondrial oxidative phosphorylation (OXPHOS) system, 22 transfer RNAs (tRNAs) involved in mitochondrial protein synthesis, and 2 ribosomal RNAs (rRNAs), namely 12S and 16S, are necessary for the translation of mitochondrial subunits. It is evident that there exists a close and functional relationship between the nuclear genome and the mitochondrial genome, approximately 1500 genes necessary for mitochondrial function are located in the nuclear genome (Schatten et al., 2014).

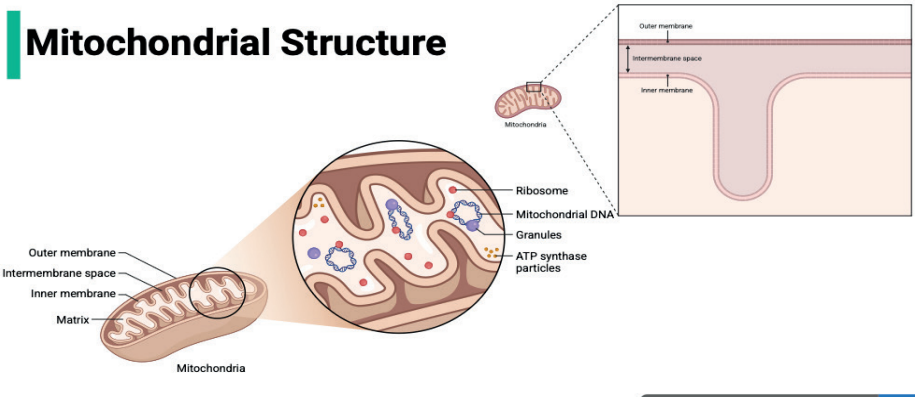
For a considerable duration, mitochondria have been designated as the cell's powerhouse. The primary rationale for this phenomenon is that, while ATP, the cellular energy unit, is predominantly produced in mitochondria, recent studies have demonstrated that mitochondria are not confined to their role in energy production (McBride et al., 2006). Mitochondria are responsible for the production of ATP, which is essential for fundamental cellular processes such as transcription, replication, and DNA damage repair. Moreover, mitochondrial metabolism contributes to the formation of various metabolic intermediates, primarily citrate and acetyl-CoA, which are necessary for important biosynthetic processes such as fatty acid, cholesterol, and steroid synthesis. A substantial proportion of these metabolites also function as signaling cofactors that directly influence chromatin structure and nuclear gene expression (Y. Xu et al., 2026). Mitochondria also play important roles in many fundamental cellular processes, including calcium homeostasis, lipid and amino acid metabolism, hemoglobin synthesis, and thermogenesis (T.-g. Zhang & Miao, 2023).

ATP is predominantly synthesized via oxidative phosphorylation (OXPHOS) in the mitochondria, with supplementary contributions from glycolysis. The continuous production of ATP is imperative for meeting cellular energy requirements and sustaining energy needs in biosynthetic processes. This process is of fundamental importance in ensuring the continuity of both catabolic and anabolic metabolic pathways (Casanova et al., 2023; Pinna et al., 2022; Spinelli & Haigis, 2018). Mitochondria are responsible for the production of approximately 90 % of the cellular adenosine triphosphate

(ATP) through a process known as OXPHOS. However, electron leakage from the respiratory chain during this process can result in the formation of reactive oxygen species (ROS) as byproducts. The ROSs in question are superoxide anion (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) (X. Xu et al., 2025). The equilibrium between the generation of ROS and the capacity of antioxidant defense systems within cells to neutralize these molecules is a pivotal factor in determining the redox state of the cell. In instances where ROS levels exceed the cell's antioxidant capacity, an occurrence known as oxidative stress ensues, which can result in damage to cellular structures and impaired function (Palma et al., 2020). However, at low physiological concentrations, ROS molecules act as second messengers, playing important roles in redox signaling and contributing to the regulation of fundamental biological processes such as cellular proliferation, differentiation, apoptosis, and stress adaptation (Ježek et al., 2020; Mironova et al., 2026). It has been demonstrated that elevated levels of ROS molecules can induce cellular damage. This process is characterized by oxidative changes in proteins (particularly the oxidation of cysteine residues), lipids (lipid peroxidation), and nucleic acids (DNA damage). Furthermore, ROS has been demonstrated to induce damage to cellular organelles and the integrity of the cell, in addition to other macromolecules, thereby precipitating various functional disorders. For instance, the hydroxyl radical (OH) is recognized as a highly reactive oxidant capable of oxidizing phospholipids and proteins found in cell membranes. This type of oxidative damage has been demonstrated to trigger various signaling cascades that activate proliferative and apoptotic signaling pathways. Consequently, these processes contribute to the disruption of cellular homeostasis and are associated with the development of pathological conditions such as neurodegenerative diseases, cardiovascular diseases, tumor formation, and cell death (Maldonado et al., 2023). Acetyl-CoA is synthesized within the mitochondria via multiple metabolic pathways. The primary source of this compound is the oxidative decarboxylation of pyruvate, a process that is catalyzed by the pyruvate dehydrogenase complex (Patel & Korotchkina, 2006; Tavoulari et al., 2023). Furthermore, the process of β-oxidation of fatty acids, ketone body metabolism, and the catabolism of amino acids such as leucine (ketogenic), isoleucine (both ketogenic and glucogenic), and valine (glucogenic) are among other metabolic sources that contribute to acetyl-CoA production (Houten & Wanders, 2010; Li & Hoppe, 2023; Matsuura et al., 2023). Furthermore, acetate can be converted to acetyl-CoA via the mitochondrial acyl-CoA synthetase short-chain family member 1 enzyme, and this contributes to the cell's adaptation to changing nutritional conditions (Bradshaw, 2021; Hlavaty et al., 2024; Pietrocola et al., 2015; Russo et al., 2023).

When all these metabolic pathways are considered together, they enable

the continuous supply of acetyl-CoA, making it a central metabolic hub that integrates nutrient availability, energy production, and biosynthetic capacity. Concurrently, acetyl-CoA functions as a pivotal acetyl donor, thereby establishing a connection between mitochondrial metabolism and nuclear epigenetic regulation. Moreover, it supplies the requisite substrates for the synthesis of fatty acids, cholesterol, and steroids (Y. Xu et al., 2026).



Figur 1. Mitochondrial structure (created with BioRender.com).

2. The Role of Mitochondria in Disease Pathogenesis

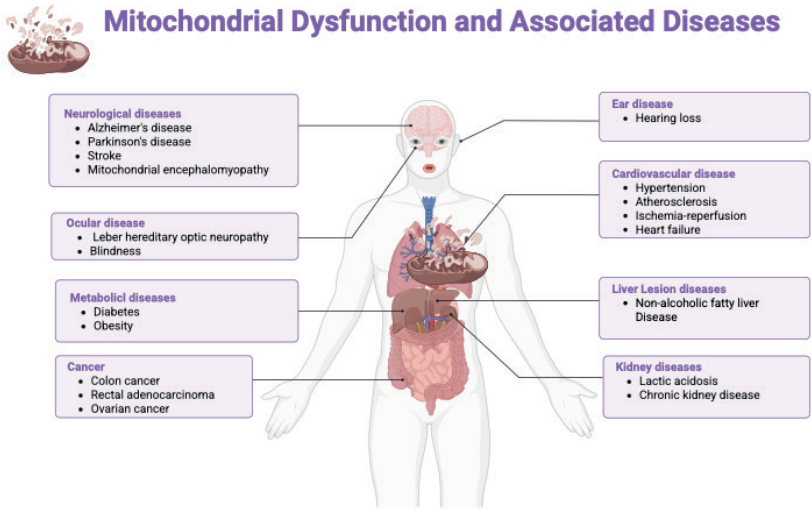
Mitochondrial diseases are genetic disorders caused by mitochondrial dysfunction (Wen et al., 2025). The identification of novel disease genes and the functional characterization of pathomechanisms related to mitochondrial energy metabolism have resulted in a steady increase in the number of genetically defined mitochondrial diseases. A review of current studies indicates that variants identified in more than 425 genes have been associated with mitochondrial disorders (Schlieben & Prokisch, 2020, 2023). Anomalies in mitochondrial function and structure are among the key determinants of neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's. Research has demonstrated that in such cases, neuronal mitochondria undergo morphological alterations, including shortening, swelling, and the formation of a mitochondrial filament structure. A recent study conducted on models of Alzheimer's disease discovered that the suppression of the mitochondrial fission factor (Mff) led to the restoration of dendritic mitochondria, thereby preventing the loss of synapses. However, it was also observed that Mff deficiency caused excessive elongation of axonal mitochondria, reducing presynaptic release and disrupting axonal branching. It is imperative to simultaneously stabilize mitochondrial balance in both dendritic and axonal compartments to ensure the maintenance of neuronal functionality under pathological conditions (Kim et al., 2026).

Metabolic syndromes, including diabetes, cardiovascular disorders, certain types of cancer, and mitochondrial diseases, have been directly linked to mitochondrial dysfunction (Leeming et al., 2026). Mitochondrial diseases are classified as rare genetic disorders that manifest considerable heterogeneity with regard to age of onset and clinical severity (Frazier et al., 2019; Gorman et al., 2016). The fundamental pathologies of these conditions are generally based on mitochondrial dysfunction, which is characterized by insufficient energy production. According to the prevailing classification system, mitochondrial diseases are categorized into two primary groups:

A. Primary Mitochondrial Diseases (PMD): These result from mutations in genes that are directly linked to OXPHOS function.

B. Secondary Mitochondrial Diseases (SMD). These originate from irregularities in mitochondrial pathways that indirectly affect the OXPHOS system.

Historically, the identification of genes associated with PMD was more common due to targeted gene sequencing studies; however, next-generation sequencing technologies have significantly accelerated the discovery of genes linked to SMD. The current catalogue of genes associated with mitochondrial diseases has surpassed 350, with a substantial proportion of these genes being classified as SMD. It has been determined that genes associated with SMD are linked to deficiencies in fundamental processes such as protein import, maturation, and quality control. These processes are critical for maintaining mitochondrial homeostasis (Baker et al., 2022; Frazier et al., 2019; Leeming et al., 2026).



Figur 2. Mitochondrial diseases (created with BioRender.com).

Mitochondrial diseases are regarded not solely as energy deficiency disorders, but also as pathway-based diseases. It is important to note that genetic defects do not invariably result in a direct reduction in ATP production, as the body possesses the capacity to compensate for diminished ATP through the process of anaerobic glycolysis. Furthermore, since mitochondria have been demonstrated to act as a buffer against these genetic lesions, environmental factors also play a critical role in triggering the diseases (McCormick et al., 2013). Quality control and stress responses utilize sophisticated quality control systems at various levels to prevent mitochondrial dysfunction and maintain balance (homeostasis) (Balsa et al., 2020; von Kleist-Retzow et al., 2007).

A. Molecular level: The present study investigates the activation of proteome control mechanisms, such as the mitochondrial integrated stress response (mt-ISR).

B. Organelle level. Mitochondria have the capacity to modify their structural configuration and spatial distribution through a series of processes, including fusion, fission, and transport, in order to acclimatize to stress.

C. Cellular level: In order to maintain a healthy mitochondrial population, mitophagy (the process of degrading damaged mitochondria) and mitochondrial biogenesis (the process of forming new mitochondria) must function in a coordinated manner.

D. Intercellular level: The process of mitochondrial transfer between cells has also been demonstrated to contribute to the maintenance of balance.

3. Mitochondrial Transplantation: A New Treatment Approach

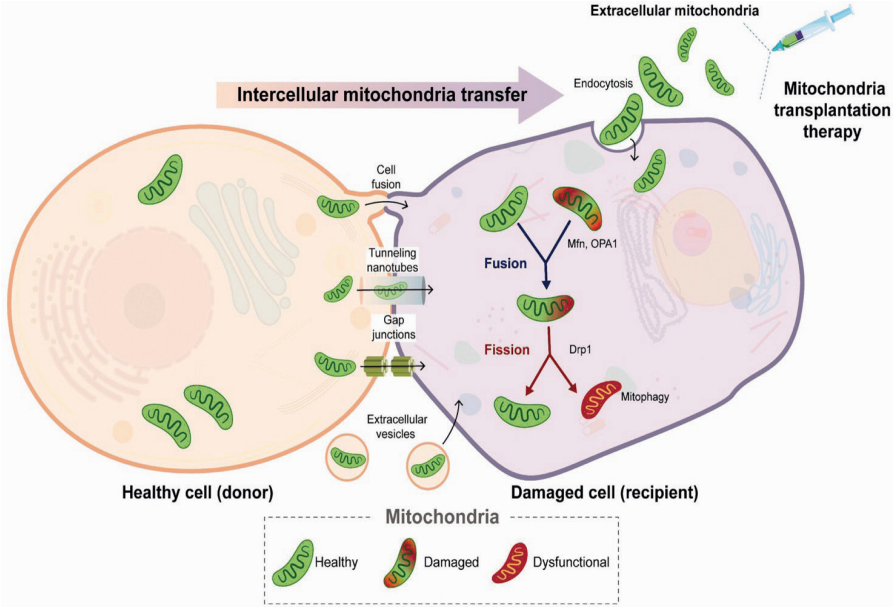
Recent studies have demonstrated that mitochondrial transplantation, a therapeutic approach aimed at restoring mitochondrial function, has yielded promising results. This approach involves the transfer of functional exogenous mitochondria to target host cells or organs. The preservation of mitochondrial activity is contingent upon the maintenance of the purity and functional integrity of the isolated mitochondria. Consequently, the mitochondrial isolation process must be executed expeditiously and under low-temperature conditions (approximately 4° C) (T.-g. Zhang & Miao, 2023). Cells transfer mitochondria to each other through four basic structural channels and methods:

Tunnel Nanotubes (TNTs): These membrane-bound, tube-shaped protrusions facilitate the connection of adjacent cells to one another. These structures are considered to represent the primary mitochondrial transfer pathway between cells (Drab et al., 2019; D. Liu et al., 2021; Qin et al., 2021).

Dendrites: Another form of membrane protrusion has been observed to form intercellular networks. It has been demonstrated that specific cell types, including osteocytes, are capable of facilitating mitochondrial exchange through the utilization of their own dendritic structures (Gao et al., 2019).

Extracellular Vesicles (Microvesicles): Formed by the outward budding of the cell membrane, these exosomes (30-100 nm), microvesicles (100 nm-1 μm), and apoptotic bodies (>1 μm) can internalize organelles such as mitochondria due to their size. This method is particularly suitable for long-distance transfers (Caicedo et al., 2017; Mittelbrunn & Sánchez-Madrid, 2012).

Naked Mitochondrial Release: Mitochondria have been observed to be released outside the cell in a naked state, thus obviating the need for a carrier or membrane-bound vesicle (Caicedo et al., 2017; T.-g. Zhang & Miao, 2023).



Figur 3. Mitochondrial transplantation mechanism (Nakano et al., 2026).

3.1 Mitochondrial Transplantation in Cardiovascular Diseases

Mitochondria play indispensable roles in the cardiovascular system because they are the energy centers of cardiomyocytes, continuously supplying ATP. Mitochondria, a critical component of eukaryotic cells, play pivotal roles in various physiological processes such as cell signaling, redox balance, amino acid and lipid biotransformation, calcium homeostasis, apoptosis, and programmed cell death. Intracellular energy balance is imperative for the survival of cardiomyocytes, which, being one of the cell

types with the highest mitochondrial content, are significantly reliant on mitochondrial oxidative phosphorylation for ATP production. Mitochondria have the capacity to rapidly adapt to changing environments in order to maintain metabolic homeostasis. In this context, mitochondrial dysfunction has been demonstrated to play a critical role in both the onset and progression of cardiovascular diseases (Huang et al., 2022; M. Liu et al., 2022; Sun et al., 2023). The field is particularly focused on cardiovascular diseases, including but not limited to ischemia-reperfusion injury, myocardial infarction, heart failure, and pulmonary hypertension (Manolis et al., 2021). Research undertaken in animal models has demonstrated that the transplantation of mitochondria can enhance energy production in heart tissue, reduce oxidative stress, and contribute to improved cardiac function. It has also been reported that this approach has the potential to reduce inflammation, prevent cell death, and support tissue repair (Sun et al., 2023). In a study addressing an innovative cellular-level approach to treating dilated cardiomyopathy a disease commonly observed in canines and characterized by weakening of the heart muscle researchers examined the therapeutic effects of intravenously transplanting mitochondria from a healthy donor to address the underlying energy production issues associated with the disease, using a case study. Subsequent to the implementation of the procedure, a swift and substantial enhancement in the heart's contractile strength and overall functionality was observed, thereby substantiating the notion that this methodology has the potential to serve as a formidable alternative to conventional therapeutic interventions (Varlik et al., 2026).

3.2 Mitochondrial Transplantation in Neurodegenerative Diseases

The functionality of neurons and the structural integrity of cells within the central nervous system (CNS) are contingent on the structure and functional status of mitochondria. Mitochondrial dysfunction has been demonstrated to result in a decline in ATP production within brain tissue, a phenomenon that has the potential to interfere with ATP-dependent neuronal activity and neurotransmitter dynamics. Consequently, the restoration of the energy production capacity of mitochondria is regarded as a significant objective in enhancing neuronal function. The transfer of mitochondria between cells has become a significant area of research, particularly in relation to central nervous system diseases. As demonstrated by experimental studies, the transfer of healthy mitochondria to damaged tissues has been shown to increase cellular energy production and contribute to the preservation of neuronal functions. Consequently, mitochondrial transplantation is regarded as a prospective therapeutic modality for the management of neurological diseases. The re-regulation of energy metabolism and support of neuronal functions through the transfer of healthy mitochondria to damaged cells is considered a promising approach for future therapeutic applications (Nakano

et al., 2026). In neurodegenerative diseases known as tauopathies, which cause cognitive decline, mitochondria isolated from healthy cells have been directly transferred to diseased cells to repair energy production impaired by protein accumulation. This transplantation has been found to increase cell viability, elevate energy levels, and reduce harmful free radicals. The enhancements observed, especially in neurons exhibiting the TAU mutation, substantiate the favorable impacts of this approach on cell renewal and neural development (Broeglin et al., 2025).

When examining the therapeutic potential of exogenous healthy mitochondrial transfer to repair cellular damage in Parkinson's disease, both *in vitro* and *in vivo* analyses have determined that it increases cell viability after transplantation and improves motor coordination and psychological symptoms in affected individuals. The research findings indicate that the transferred mitochondria offer neurological protection, primarily by modulating inflammatory responses and cell death pathways (Si et al., 2026). A study has thoroughly investigated the cellular mechanisms of brain damage caused by oxygen deprivation in newborns and the importance of mitochondria, which play a central role in this process. The study found that oxygen loss disrupts mitochondria, the cell's energy center, leading to an increase in harmful free radicals and triggering nerve cell death. Recent studies have indicated that therapeutic interventions aimed at enhancing mitochondrial function present a potentially efficacious medical approach for the management of brain injuries in infancy (Frańczak et al., 2026). Healthy mitochondria obtained from muscle tissue were directly transplanted into the brain cavities with the aim of repairing brain tissue damaged after an ischemic stroke. Researchers achieved a reduction in cell death and oxidative stress, thereby diminishing the size of the damaged area in the brain, through the utilization of this autologous mitochondrial transplantation method. This innovative method has been shown to not only prevent tissue loss but also to trigger nerve cell development, thereby accelerating the recovery of cognitive and physical functions. Consequently, the increase in the amount of live mitochondria in the cerebrospinal fluid is defined as a critical indicator of the success of the post-stroke recovery process (Norat et al., 2020; Z. Zhang et al., 2019).

3.3 Mitochondrial Transplantation in Cancer

Increased lactate production due to elevated glycolytic flux lowers extracellular pH, leading to acidosis in the tumor microenvironment. This contributes to immune evasion, cancer invasion, metastasis, and even resistance to radiation and drugs. In the context of examining the effects of the glycolysis-priority survival strategy, otherwise known as the Warburg Effect, on tumor development, it is emphasized that cancer is not merely a disease based on dysfunctional mitochondria; rather, mitochondrial transfer and

transport processes play a critical role in cancerous tissues in terms of gaining resistance or creating treatment targets (Cruz-Gregorio et al., 2023). A study investigates how mitochondria transferred through cell-to-cell contact trigger cancer spread and examines the complex nature of this process. The objective of the present study was to illuminate the effects of these organelles on breast cancer by means of transferring them using an artificial intervention termed mitochondrial transplantation. The findings of the study demonstrated that the external regulation of mitochondrial functions did not demonstrate significant anti-tumor potential in terms of halting tumor development (Chang et al., 2019).

Mitochondria have been demonstrated to play a critical role in the progression of lung cancer and treatment resistance. This study examined how these cells are metabolically reprogrammed, and analyzed the potential of mitochondrial inhibitors to disrupt the function of these organelles as a strategic treatment method. The mechanisms of different drug classes and the toxicity risks encountered in clinical trials were systematically examined, with a particular focus on targeting resistance to existing therapies. The study concludes that targeting mitochondrial sensitivities represents a comprehensive and promising roadmap for overcoming the fundamental obstacles in modern lung cancer treatment (Park, 2026). A recent study investigated the potential of mitochondrial transfer between different species to slow the spread of cancer cells. In the course of the research, the scientists transferred mitochondria from healthy blood vessel cells to aggressive melanoma cells. This resulted in a fundamental change in the cell's energy production and structural form. Consequently, the invasive capacity and viability of cancer cells diminished, while the fundamental genetic signals triggering tumor growth were suppressed (Kuo et al., 2026).

3.4 Mitochondrial Transplantation in Other Diseases

In a study investigating the combined use of curcumin and mitochondrial transfer derived from mesenchymal stem cells (MSCs) for the treatment of age-related macular degeneration (AMD), researchers observed that this dual therapy combats cellular stress and improves the functioning of genes critical for eye health in an *in vitro* retinal model. Specifically, the combination of these methods strengthens biological markers that could help preserve vision by reducing oxidative damage and inflammation in cells (Kılıç et al., 2025). The study investigated the therapeutic potential of extracellular mitochondrial transfer against osteoarthritis, a disease characterised by cartilage tissue degeneration. The study indicated that using healthy mitochondria obtained from stem cells to restore the function of damaged cartilage cells increased energy production in these cells and reduced inflammation. The experimental findings demonstrated that this method has the capacity to restrict cell death and promote type-2 collagen production, a process that is imperative for

maintaining optimal joint health (Altuntaş et al., 2025). A study was conducted to examine the effects of exercise on mitochondrial balance in skeletal muscle, with the objective being to investigate how the cell's energy centers respond to physical stress. It was observed that the cellular stress induced by strenuous exercise activates a protective and reparative mechanism known as the mitochondrial unfolded protein response (UPRmt). The experiments revealed that acute exercise initiates this genetic signaling pathway by increasing free radical production, thereby enhancing mitochondrial activity and strengthening cellular adaptation. The present study set out to investigate the potential effects of isolated mitochondrial transplantation from mesenchymal stem cells in mitigating the effects of ischemia-reperfusion damage. Such damage is frequently encountered in surgical procedures, such as kidney transplantation. The study observed that direct mitochondrial transplantation into damaged kidney tissue triggered cellular renewal and proliferation at the cellular level, while also reducing apoptotic markers that lead to tissue death. By establishing a direct correlation between mitochondrial dysfunction and renal damage, the researchers demonstrate the critical importance of this method in the healing process (Kubat et al., 2021). In a study examining the effectiveness of exercise and mitochondrial transplantation methods to prevent skeletal muscle loss caused by doxorubicin, a common chemotherapy drug, researchers observed that both interventions, when applied individually or in combination under *in vitro* conditions, protected muscle tissue by reducing cell death and inflammation. The most comprehensive protection was provided by a combination of mitochondrial transplantation and exercise, as evidenced by the triggering of mitochondrial biogenesis and the prevention of oxidative damage, thereby strengthening the structure of muscle fibers (Kubat et al., 2024). It is evident that the body's innate capacity for self-repair is an energy-consuming process, thus underscoring the pivotal function of mitochondria, the energy powerhouses of cells, in this healing mechanism. In a study conducted by a research team working in this field, the effects of transplanting healthy mitochondria on tissue in the treatment of chronic wounds were examined, and it was clearly observed that injecting mitochondria taken from muscle tissue reduced the size of the wound and accelerated new tissue formation (Taner et al., 2024). When the critical role of MSCs in improving diabetes-related kidney damage was examined through *in vivo* analyses, it was determined that the direct transfer of healthy mitochondria obtained from these cells to the kidney rapidly repaired tissue structure by reducing oxidative stress in the cells and re-stabilized the fundamental transport proteins that maintain the kidney's filtration function (Konari et al., 2019).

In conclusion, these findings provide a scientific basis for future clinical applications by offering an innovative and promising strategy for combating mitochondrial disorders.

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Chapter 4

THE EFFECT OF HEAT AND DROUGHT STRESS ON PLANTS

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INTRODUCTION

Since the formation of the Earth, climate has tended to change due to natural causes. However, especially after the mid-19th century, various human activities have led to the disruption of the natural balance of climate change. Gases released into the atmosphere due to many reasons such as fossil fuel use, industry, transportation, pesticides, indiscriminate hunting, deforestation, unplanned urbanization, domestic waste dumped into nature, nuclear activities, and population growth have increased the natural greenhouse effect. The term "global warming" describes how the greenhouse effect has caused the Earth's surface temperature to rise. The number of hot days and nights is increasing, the number of cold days is decreasing, sea levels are rising, rainfall is decreasing, water resources are being depleted, drought is occurring, agricultural production is declining, biodiversity is declining, species changes are occurring, and some species are going extinct because of global climate change.

Various studies indicate that the world and our country are expected to face hotter and drier conditions in the future. Drought and extreme heat are among the leading factors causing significant yield losses in agricultural production. Temperature affects all stages of plant development, including seed germination, seedling formation, flowering, pollination, fertilization, fruit formation, and plant yield. Every plant has a preferred temperature range for optimal growth and development. Both below and above the ideal temperature have detrimental effects on growth and development. All parts of the plant are adversely affected by heat stress. Heat's impact on pollen, however, is crucial since it influences both plant reproduction and yield.

The severe drought and heat events expected due to global warming indicate the need for serious research into increasing plant tolerance. Therefore, it is necessary to identify specific plant processes affected by heat and drought and to investigate plant tolerance mechanisms. Identifying resistant genotypes in plants can help improve the resistance of other plants. The impact of temperature on pollen germination has been the subject of numerous studies in recent years. These studies showed that germination was reduced in all pollens exposed to or subjected to high daytime temperatures, both *in vivo* and *in vitro*. However, several consequences have been observed, including reduced or slowed tube growth, a decrease in the number of tubes reaching the ovary, fertilization inefficiency, reduced seed set and fruit set, failure in flowering and fruit yield, and a decrease in ATP energy and carbohydrate concentration. Current findings suggest that drought stress has a detrimental influence on pollen germination, despite the paucity of research examining this relationship. Drought reduces pollen viability, leading to poor fertilization.

The purpose of this study is to ascertain how plants, is affected by heat

and drought stresses, and thus to reveal the negative effects of global warming on various plants, especially on the pollen germination process.

CLIMATE CHANGE

The term "climate" refers to the average of long-term meteorological observations of a location, including temperature, humidity, air pressure, wind, precipitation, and type of precipitation. The atmosphere, land surfaces, snow and ice, seas and other bodies of water, and living things are all parts of the complex and dynamic climate system. This system undergoes steady change over time because of shifting external circumstances or internal dynamics. "Changes in the average state and/or variability of the climate over decades or longer, whatever the source" is the definition of climate change (Turner et al., 2014). Over the past 400,000 years, the climate has exhibited a periodic cycle of ice ages and warm periods and has been remarkably stable over the last 8,000 years, with only minor temperature fluctuations. However, today, climate change is occurring not due to natural causes as it has since the formation of the Earth, but due to factors such as fossil fuels, improper land use, deforestation, and increased emissions of gases into the atmosphere resulting from industrial development (Butler, 2018).

Short-wavelength radiation from the sun reaches our planet's surface and is converted into heat, warming the Earth. The Earth's surface and atmosphere warm more than they should because to the absorption of greenhouse gas molecules in the atmosphere, while some of this radiation is reflected into space as long-wavelength infrared radiation. This phenomenon is called the greenhouse effect (King, 2005). The Earth's average temperature would be -18°C without the greenhouse effect, making it an essential mechanism.

The term "global climate change" describes the rise in Earth's average surface temperature and the ensuing climate changes brought on by an increase in the natural greenhouse effect brought on by greenhouse gases [carbon dioxide (CO_2), methane (CH_4), chlorofluorocarbons (CFCs), and ozone (O)] released into the atmosphere because of human activity (Dellal et al., 2011).

According to projections, global surface temperatures will rise by 1.4 to 5.8 degrees Celsius by the end of the twenty-first century and by roughly 0.6 degrees Celsius from the late nineteenth century to the present (Houghton, 2001). Additionally, it is predicted that severe occurrences like hot days would happen more frequently in future climates and that the diurnal temperature range will shrink (Dai et al., 2009). The duration of the growing season and threshold temperatures are two factors that determine whether a crop is suitable for a certain area. Temperatures above optimal and high levels on a daily or seasonal basis will become a major limiting factor in crop output if they occur during important periods of plant development (Kakani et al., 2005).

Climate Change Worldwide and Its Effects on Living Organisms

According to the Intergovernmental Panel on Climate Change (IPCC) 2007 Report, the frequency of cold days, nights, and frost events is decreasing in terrestrial areas. Hot days and nights are becoming more frequent. Heat waves are occurring in most terrestrial areas. Extreme sea level rises are increasing. Both the frequency of severe tropical cyclones in the North Atlantic and the areas affected by drought worldwide have grown since the 1970s. Due to climate change, the species composition is changing in many parts of the world, including Europe, and species are becoming extinct at a rate 100–1000 times faster than what is considered normal (Bakkenes et al., 2002). Studies have shown a high correlation between changes in plant composition and the current climate change (Uzunoğlu et al., 2015).

By 2050, it is expected that species distribution will be significantly affected in many countries such as Spain, France, and Algeria, and that they will lose 80% of their diversity (Bakkenes et al., 2002). At the same time, it is predicted that 15–37% of all species on Earth will become extinct (Clarke, 2014; Uzunoğlu et al., 2015).

The average global temperature is expected to rise by 1-3.5°C by 2100, according to modeling studies used to understand global climate change. This would cause regional extreme temperatures, floods, and widespread and severe droughts all over the planet (Öztürk, 2015; Öztürk et al., 2021). A 3°C increase in temperatures, within the predicted range for 2100, will cause species distribution to shift 300-400 km north or to 500 m higher altitudes in temperate regions. This change in species distribution indicates that species will concentrate in certain regions, and those that cannot adapt to environmental changes will face the threat of extinction (Tariq et al., 2024).

Global warming is known to affect the flowering periods of plants. For example, in some parts of North America, lilacs (*Syringa sp.*) bloomed 1.8 days earlier each decade between 1959 and 1993 (Schwartz and Reiter, 2000), and honeysuckle (*Lonicera sp.*) bloomed 3.8 days earlier each decade in the United States' west (Cayan et al., 2001). Since 1980, oak trees in the Netherlands have been seen to open their buds earlier because of a 2˚C rise in temperature (Uzunoğlu et al., 2015).

Climate Change in Turkey and Its Effects on Living Organisms

Turkey is one of the nations that will be most impacted by the effects of global warming because of its geographic location. Reducing the adverse effects of climate change and to be ready for its repercussions, it is necessary to predict how observed climate changes and trends will unfold in the future and to determine the impacts of these changes on natural and human systems. Future atmospheric quantities of compounds that upset the Earth's

radiative balance, including greenhouse gases and aerosols, are predicted by emission scenarios (Moss et al., 2010). Developed within the Intergovernmental Panel on Climate Change (IPCC) with the participation of expert scientists from all sectors, these scenarios consider various assumptions regarding population growth, energy use, economies, technological developments, and changes in agriculture and land use. Adjustments are made to the scenarios considering evolving technology and changes in the components under consideration. Human-induced (anthropogenic) climate change scenarios, which primarily focus on radiative forcing and the climate system's response to this forcing, are one of the most important components of the IPCC's work (Reinman, 2012).

The 4th Assessment Report of the IPCC is based on simulations of the high-emission scenario (A2) model., predict that annual temperatures in Turkey will increase by 3.1 - 5.2 °C by the end of the century compared to today. According to the RCP4.5 scenario, the average annual temperature in Turkey is expected to increase by 1.5 - 2.6 °C between 2016-2099. According to the RCP8.5 scenario, the average annual temperature in Turkey is expected to increase by 2.5 - 3.7 °C between 2016-2099.

According to annual total rainfall projections, decreases in rainfall are predicted across Turkey in the latter part of the century (2081-2100). While a general decrease in rainfall is expected, there is no continuous increasing or decreasing trend; rather, rainfall irregularities are showing an increasing trend. According to the RCP4.5 scenario, the average annual total rainfall anomaly in Turkey is expected to decrease by 3% to 6% during the 2016-2099 period. According to the RCP8.5 scenario, Turkey's annual total rainfall anomaly is predicted to shift by an average of +3% to -12% between 2016 and 2099 (Change, 2007).

The phenological stages of field and garden crops are believed to have moved forward due to the considerable temperature rises that Turkey saw after the 1990s. Although a 1°C increase in temperature does not significantly alter people's daily routines, it can alter phenological phases and is equivalent to 60 day-degrees in two months for plants. According to a climate index study, Turkey's growing season lengthened by 21 days on average every century (Şensoy et al., 2013). Grain fullness and density, number of grains per spike, and grain weight in cereals will all suffer from a shorter growth season, although field crops and viticulture can benefit from longer growing seasons through species selection and rotation. Fruit trees that flower early may be more susceptible to harm from late frost (Türkoğlu et al., 2012).

Turkey's agricultural, forestry, and water resources may be negatively impacted by climate change, especially in the semi-arid and semi-humid regions that are at risk of desertification (Central Anatolia, Southeastern Anatolia,

Aegean and Mediterranean regions) (Türkeş, 1998). There is compelling evidence that the main causes of the mass tree die-offs and pest infestations that have developed in Turkish forests in recent years are drought, air pollution, and acid rain. These effects negatively impact the biodiversity, gene reserves, and carbon sequestration capacity of forests (Aguilos et al., 2025).

A significant issue is climate change, particularly for indigenous species. The abundance of forest species in Turkey, especially endemic plant species, suggests that climate change would have a significant influence. Another plant group potentially at risk in our country are geophytes, which are bulbous plants. Nearly 300 of the 600 species of bulbous plants are endemic. The decline in winter rainfall and, more significantly, the rise in winter temperatures have a detrimental effect on these plants, which bloom in the winter and early spring, as well as the biological chain in which they are found (Uzunoğlu et al., 2015).

STRESS IN PLANTS

Stress is the term used to describe alterations in plants that have a detrimental impact on growth and development, lowering the quality and quantity of the final products and, in extreme cases, resulting in the death of plants and plant organs. Both biotic and abiotic environmental conditions can cause stress in plants. Optimal environmental conditions are those that do not result in stress; in this instance, plants have fully adapted to their surroundings. Stress factors are defined and classified in different ways by most researchers (Mareri et al., 2022). In the most general terms, abiotic stress factors; heat, cold, drought, flood, heavy metals, and salt and biotic stress factors; pathogen, insect, and herbivore attacks (Ameen et al., 2024). Depending on how well a plant has adapted genetically to its surroundings, different stressors can produce different amounts of harm. Stress factors lower plant productivity by 65% to 87%, depending on the crop. Plants will respond differently depending on the type, severity, duration, presence of a single or multiple stress source, frequency of exposure to stress, and developmental stages of the stressor (Zhang et al., 2022).

TEMPERATURE STRESS

The temperature at which each plant grows and develops most effectively is called the optimum temperature. Temperature stress occurs at temperatures above the optimum temperature. One of the abiotic stress conditions that restricts growth, and development is high temperature stress, particularly when it rises above the ideal growth temperature. This is because it inhibits photosynthesis, damages cell membranes, and causes senescence, or cell aging, which results in cell death. At low temperatures, biological membranes stiffen, and more energy is required for biochemical functions to be carried out (Zafar et al., 2024)

Although the productivity of ecosystems is determined by the combined effect of all environmental factors, one of the most significant variables influencing plant metabolism is temperature. In general, each plant species has its own maximum, minimum, and optimum temperature values. Hot and cold, depending on their intensity and duration, affect metabolic activity, growth, and development, thus limiting the distribution of plant species across countries and regions (Chen et al., 2026).

Effects of High Temperatures

Stress from high temperatures impairs a plant's physiological and biochemical processes, which reduces its growth, yield, and quality. To stop water loss, stomata close in plants under stress from high temperatures. It causes a decrease in chloroplast photochemical activity. High temperatures negatively affect photosynthesis, and consequently, growth and development. Substrates are depleted through respiration. It causes necrotic bruising in the stem (González-García et al., 2023). Yellow spots appear on leaves, fruits, and pine needles. It leads to the denaturing of proteins and the loss of enzyme activity in plants. It causes changes in cell structure and cell functions. The movement of water, ions, and organic solvents decreases. The effect of temperature on the cell membrane is also very important. Due to high temperatures, damage occurs in the protein and lipid structure of membranes. Metabolic rate imbalances occur. Premature aging may occur along with accelerated growth. High temperatures cause heat death in plants. The occurrence of heat death is closely related to the duration of exposure to high temperatures (Qian et al., 2025).

The Role of Cell Membranes in Heat Stress

The first places in plants where high temperatures cause physiological harm are cellular membranes. A popular plant physiological metric for examining a plant's temperature tolerance is cell membrane thermostability. Cotton cultivars with high temperature tolerance have been effectively screened using it (Kakani et al., 2005). Under high-temperature conditions, cotton plant cultivars with high membrane thermostability have produced more seeds during seed filling and flowering (Manan et al., 2022).

Strategies Developed in Plants for Temperature Tolerance

Heat stress resistance is defined as the ability of some genotypes to perform better than others when exposed to the same heat stress. Heat resistance mechanisms are divided into two categories. First one is heat-avoidance. This is a genotype's capacity to disperse radiation energy to keep plant temperature increases from becoming stressful. It is the most important energy dissipation mechanism (Bai et al., 2023). The second one is heating tolerance. This is the ability of some genotypes to withstand/perform better than others in a heat

stress environment when their internal temperatures are compared (Wang et al., 2025).

Recent studies have found that plants produce certain special proteins in high-temperature shock. While normal protein synthesis decreases, it leads to the formation of a series of new proteins called Heat Shock Proteins. This phenomenon occurs when the ambient temperature rises 5°C above the temperature required for optimum development. These proteins are formed in the cytoplasm, mitochondria, and chloroplasts and are classified under different groups (Rivero et al., 2022).

DROUGHT STRESS

One of the main factors limiting agricultural productivity and having a significant impact on crop yield is drought. Furthermore, as one of the primary factors impeding plant growth, drought can impair stomatal movement, respiration, and photosynthesis, which in turn impacts plant growth and physiological metabolism. Plants respond to drought stress by triggering their drought response mechanisms, which include morphological and structural alterations, the activation of genes resistant to drought, hormone synthesis, and the production of osmotic regulating chemicals (Yang et al., 2021).

Drought is defined differently by various disciplines, leading to confusion in its definition. The World Meteorological Organization (WMO) defines drought as a sustained and ongoing drop in precipitation. The United Nations Convention to Combat Desertification and Drought states (UNCCD), drought is a natural phenomenon resulting from a significant decrease in rainfall compared to recorded normal levels, causing serious hydrological imbalances that negatively affect the production systems of Earth's resources (Akhtar-Schuster et al., 2022).

According to the United Nations World Agriculture Organization (FAO), drought damage is defined as the percentage of years in which crops are damaged due to reduced moisture (Örs and Ekinci, 2015). Drought in agriculture refers to the amount of water that a planted crop can absorb via its roots during its growth stage, not the total amount of rainfall that occurs during the year. Water shortages throughout the growing season cause major losses for plants in terms of growth and, most importantly, yield (Cao et al., 2022).

Turkey's geographic location influences the dominant atmospheric masses, which in turn define the country's climate. The nation's climate is significantly diversified by its unique position and physiographic features. Most of the Turkey is influenced by the Mediterranean Macro Climate, which is common on the western shores of continents with comparable latitudes. This climate, which is characterized by an annual water deficit and soil moisture shortage, includes dry, semi-arid, and semi-humid conditions found across

a large portion of Turkey. This classification is supported by several climate classification studies carried out in Turkey. Furthermore, research based on climate change scenarios predicts that Turkey's future climate would see an increase in the severity, frequency, and duration of drought occurrences (Serkendiz et al., 2024)

Drought's Physiological and Metabolic Impact on Plants

The degree to which plants are affected by drought, which varies in severity depending on the genotype, depends on the metabolic alterations—that is, the physiological and biochemical reactions—that the genotype undergoes under stress (Örs and Ekinci, 2015). When cultivated in the same climate for extended periods of time, plants can modify their growth and development processes to reduce damage from environmental changes and even to become less impacted by them. Depending on the intensity and length of the water stress, plants can drastically change their metabolisms and even their life cycles when they experience drought (Oguz et al., 2022).

Growth and Development of Plants

During a time of water shortage, a plant's developmental stage is determined by how drought stress affects plant growth and development. Drought conditions also alter a variety of physiological traits that govern plant yield. The sensitivity of plants to water scarcity is most pronounced during the generative stage. According to research by Farooq et al. (2009), severe drought conditions occurring during the developmental stage when seed formation begins lead to yield losses of up to 95%. Water scarcity, especially during the flowering stage, leads to sterility in plants (Öztürk, 2015).

The length of the water shortage determines how the growth of the plant's changes. To obtain more water during the early phases of drought, the plant reduces the elongation of its stems and promotes the growth of roots. The development of the stem and roots stops, the area and quantity of leaves decreases, and some leaves may even turn yellow and fall off if dry conditions last long enough to harm the plant. The stoppage of cell division and expansion in the shoot and root meristems is the cause of the decline in plant growth. The reduction in the rate of photosynthesis brought on by a lack of water is directly linked to the stoppage of cell division or growth (Anjum et al., 2011).

According to Bray (1997), a plant will attempt to recover by decreasing water loss or increasing water intake when it is unable to get the water it requires from its root zone and this scenario starts to produce stress. The first result that will occur in the plant is the loss of turgor (Barlow et al., 1980). The leaf cells experience plasmolysis and wilt because of the loss of turgor and the inability of the plant roots to replace the water lost by transpiration from the leaves. When compared to plants that have finished developing without wa-

ter stress, plants that have finished developing under drought stress typically have a smaller volume (Öztürk, 2015).

Photosynthesis

Narrowing or closing stomata to stop water loss is the first alteration seen in plants that feel water stress (Osakabe et al., 2014). The amount of gaseous carbon dioxide that enters plant leaf tissue through open stomata is correlated with the rate of photosynthesis. Water loss by transpiration is another consequence of open stomata. Reducing water loss through transpiration, plants quickly narrow or close their stomata during drought circumstances (Öztürk, 2015). Hydraulic cues (leaf water potential, cell turgor) and chemical signals (abscisic acid) are the two primary causes of plant stomata closure during drought (Kalefetoğlu and Ekmekçi, 2005). Due to the closure of stomata, carbon dioxide uptake decreases, leading to a reduction in the plant's photosynthesis rate. Since carbohydrate molecules and energy used in plant growth are produced through photosynthesis, this decrease is a factor affecting plant growth and development. Under drought stress, another effect of stomatal closure, particularly in the leaves and the decrease in leaf water content, is the increase in leaf temperature and the resulting damage to membrane systems, leading to cell death (Farooq et al., 2009). Oxidative damage to chloroplast lipids, pigments, or proteins may be the cause of non-stomatal photosynthetic limitation. Variations in relative water content (RWC) and ambient light intensity also have an impact on plants' ability to photosynthesize. (Kalefetoğlu and Ekmekçi, 2005).

Osmolytes

Water potential, or turgor pressure, in plant cells is also changed by drought. To guarantee that plant cells are not adversely impacted by water stress, osmotic equilibrium is essential. Therefore, in response to drought stress, plants produce and store a class of soluble compounds known as "osmolytes" in their cells, which contribute to the preservation of cellular turgor balance. These compounds may belong to several classes, such as organic acids, polysaccharides, betaine, free amino acids such asparagine, proline, and glycine, and others. Osmolytes, which oversee preserving water balance, don't immediately make plants more drought tolerant. However, because they balance leaf water pressure, they increase stomatal conductivity, ensure the continuity of photosynthesis, and thus aid growth (Öztürk, 2015).

Plants are temporarily resilient to drought circumstances due to the preservation of water balance by osmolytes and the continuing of cellular metabolic processes, albeit in limited amounts. In cases of prolonged stress, osmolyte accumulation is insufficient to compensate for turgor loss due to water deficiency. Since proline is the first free amino acid to accumulate when drought conditions occur, its intracellular concentration is a frequently used

measurement in research studies to indicate that plants are experiencing water scarcity under experimental conditions. The primary intracellular function of proline is to protect membrane systems and protein structures through the compounds they form by inhibiting lipid oxidation. But according to new research, proline may also play a part in signal transmission and be a crucial free amino acid that controls gene expression levels, cell division or death, and mitochondrial function (Ghosh et al., 2021).

Abscisic Acid

The signaling processes of drought and salinity stress are nearly the same because salinity stress, which is brought on by an increase in soil salt content, similarly impacts water uptake by roots, resulting in a drop in water potential. Abscisic acid (ABA), cytokinins, ethylene, and malate are known to transfer the perception of water deprivation from the roots to the stem (Anjum et al., 2011). ABA alters potassium ion (K^+) flow, causing stomatal closure, one of the changes plants undergo under drought. According to studies, abscisic acid actively regulates the mechanism of photosynthesis under drought stress by lowering the amount of chlorophyll synthesis, altering the structure and function of chloroplasts, and influencing the accumulation, transport, and distribution of photosynthetic products through a few as-yet-unidentified factors (Dolferus, 2014). Additionally, abscisic acid buildup is known to alter gene expression and to be crucial for the reorganization of metabolism under drought stress. Temporary elevations in ABA cause a variety of alterations, including suppression of energy expenditure pathways, accumulation of protective and compatible soluble proteins, induction of gene expression, and elevated antioxidant levels (Bartels and Sunkar, 2005).

Oxidative Damage

Free radicals, including active oxygen species [superoxide molecule (O_2^-), singlet oxygen (O), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), are formed when plants are stressed by dryness. Free radicals are extremely reactive compounds with unpaired electrons. These radicals can also be formed in the plasma membrane, mitochondria, and ER membranes (Kalefetoğlu and Ekmekçi, 2005).

According to Cabello et al. (2014), reactive oxygen species (ROS), which build up during stressful situations, are really a normal result of cellular metabolism and are crucial for signal transduction processes. However, by causing lipid peroxidation, protein decrease, and DNA destruction, excessive buildup might result in cell death. Therefore, a key component of plants' defense against stress conditions is lowering and minimizing the buildup of reactive oxygen species created during drought stress. Plants use enzymatic (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase) or non-enzymatic (glutathione, ascorbate, tocopherols, and carotenoids) antioxidant

molecules to fight oxidative stress brought on by the buildup of reactive oxygen species. Enzymatic antioxidant molecules reduce reactive oxygen species, preventing their accumulation, whereas non-enzymatic antioxidant molecules primarily protect photosynthetic membranes (Öztürk, 2015).

Additional Functional Molecules Involved in Drought Stress

Other important functional proteins involved in how plants react to drought stress include water channel proteins, heat shock proteins acting as chaperones, protheses, late embryogenesis proteins, MAP kinases, and the plant AP2/EREBP and bZIP transcription factors. The amount of water channel proteins increases during drought stress. The effective distribution of water within cells and between cells and tissues in plants during times of water scarcity is largely dependent on the accumulation of these water-selective proteins found in plasma and vacuole membranes as well as the control of the activity of already-existing proteins through processes like phosphorylation (Liu et al., 2020).

Because they were initially discovered amid heat stress, a family of proteins known as "heat-shock proteins" (HSPs) function as molecular chaperones. While heat shock proteins are responsible for ensuring that proteins fold post-translationally and assume structures suitable for membrane transport under non-stress conditions, in stressful situations such as drought and heat, they prevent the aggregation of denatured proteins and help aggregated proteins regain their functional structures (Rahman et al., 2022).

Under drought stress conditions, the carbohydrate metabolism of plants is also affected. Cellular and tissue concentrations of simple carbohydrates such as fructose and glucose change during responses to drought stress. Sugar molecules produced by photosynthesis also play a role in signal transduction between tissues. This change in the concentration of simple carbohydrates is known to be responsible for regulating photosynthesis metabolism throughout the plant. It is also thought that sugar molecules activate the enzymatic and metabolic antioxidant defense mechanisms of oligosaccharides (Wang et al., 2022).

The best known of these proteins are those belonging to the dehydrin and LEA families (this term describes a type of protein that is abundant in the late stages of embryo formation). Both families of proteins, which play important protective roles in drought stress, share the common characteristic of being low molecular weight, soluble molecules. Although LEA proteins were first identified in seed embryos, their high water-binding capacity due to their hydrophilic nature is thought to contribute to their protective role in plant stress mechanisms (Szlachtowska and Rurek, 2023)

Proteins that are expressed under water stress circumstances and are believed to be involved in the restructuring and/or maintenance of cellular metabolism are the most significant factor in plant responses to drought stress. Proteases facilitate protein conversion during drought by degrading old proteins and enzymes so that new ones can be synthesized. The fundamental signal transduction initiating transcriptome rearrangement in the abiotic stress response begins with the mitogen-activated protein kinase (MAP kinase) pathway, a family of receptor-type kinases. MAP kinases are a highly conserved family of enzymes that are essential for transducing extracellular signals into the cell genome. Often, phosphorylation of MAP kinase results in the kinase leaving the module and translocating to the nucleus. Here, MAPK activates the expression of specific genes involved under drought stress by activating and phosphorylating transcription factors (Ding et al., 2024).

MicroRNAs are another class of functional molecules that are essential for reorganizing gene expression in drought stress. According to research, plants have many distinct miRNAs that control gene expression when they are under drought stress (Cabello et al., 2014). Information about the variability of miRNAs based on the plant, stress dose, and circumstances is lacking because there aren't many research available (Ahmad et al., 2022).

Protection Mechanisms of Plants Under Drought Stress

Vegetative tissues have evolved two primary defense mechanisms against drought stress: stress tolerance and stress avoidance. Succulent plants that store water in their tissues and desert ephemerides that only live as latent seeds during the dry season are examples of stress avoidance techniques. Stress-tolerant plant groups can withstand much more severe drought stress by triggering their defense mechanisms, whereas stress-avoiding plants can only withstand moderate drought stress (Pamungkas and Farid, 2022).

TEMPERATURE'S IMPACT ON POLLEN TUBE GROWTH AND GERMINATION

Important variables impacting pollen germination and pollen tube length, which in turn affect fruit set and yield, include temperature, germination time, and plant type. In Upland (*Gossypium hirsutum*) and Pima (*G. barbadense*) cotton (Reddy et al., 1992), *Brassica napus* (Young et al., 2004) was exposed to daytime temperatures of >30 °C for about 13 hours, and tomato (*Lycopersicon esculentum*) was exposed to temperatures of >28 °C for 12 hours (Sato et al., 2002). These conditions were found to reduce fruit set. Daytime temperatures of 30 °C for 16 hours (Saini and Aspinall, 1982), 38 °C for 16 hours (Mitchell and Petolino, 1988), 6 hours (Matsui et al., 1997), 12 hours (*Phaseolus vulgaris*) at >28 °C (Prasad et al., 2002), and 12 hours (*Arachis hypogaea*) at >28 °C all decreased seed set in wheat (*Triticum aestivum*) (Prasad et al., 2011).

According to Weaver and Timm (1988), the absence of fertilization under high temperature stress may be explained by pollen's greater sensitivity to high temperatures than female reproductive systems. According to recent research, high temperatures impede micro and megasporogenesis and decrease fruit set (Young et al., 2004).

Because anther components degrade, Young et al. (2004) planted tomato plants at 32/26 °C 0–15 days prior to antecedent fruiting. In crossbreeding trials, pollen-producing plants exposed to high temperatures (35 °C for 4 hours a day) displayed an 88% decrease in fruit set, highlighting the significance of pollen in fruit set. Fruit set dropped by 37% when high temps were applied to emasculated receptor plants. Burke et al. (2004) found that the cotton cultivar Gregg 65, which is grown in a greenhouse, germinates pollen best at 28°C. According to Zinn et al. (2010) and Hedhly et al. (2009), pollen growth and function might be particularly vulnerable to high temperatures during reproductive processes. For instance, persistent exposure to high temperatures during the meiotic phase of microgametophyte growth led to poor pollen germination and seed set in *Sorghum bicolor*, according to a study by Jain et al. (2007).

High daily temperatures significantly reduced the amounts of ATP and total soluble carbohydrates in cotton pistils, according to Snider (2015). Total soluble carbohydrate content decreased by 20.3% because of heat stress, and ATP concentrations in heat-stressed pistils were almost 55% lower than those in pistils kept at optimal temperature during the sampling period. The net photosynthesis of leaves was significantly reduced by high daily temperatures. The fixation rates for leaf drop (10.7% μmol CO_2 $m^{-2}s^{-1}$) grown under ideal daily temperature circumstances were around 16.8% higher than the net CO_2 fixation rates (8.9 μmol CO_2 $m^{-2}s^{-1}$) discovered under high daily temperature settings ($P < 0.0001$). This study found that low reproductive success is primarily caused by lower *in vivo* pollen tube growth, as indicated by a drop in the pollen tube/ovule ratio. Additionally, they concluded that the energy needs of expanding pollen tubes cannot be sufficiently satisfied under heat stress since pollen tube growth is decreased along with the amount of ATP and carbohydrates in cotton pistils (Snider and Adegbenro, 2025).

The relationship between temperature and growth regulators in both shoot initiation and proliferation was examined during the *in vitro* propagation of *Narcissus triandrus* from twin scales and, the results showed how crucial the explants' culture temperature was. The behavior of tapetum cells and the structure of the pollen grain wall at 18°C were the most noticeable differences between normal and aberrant flowers in *N. triandrus* at 25°C. The pollen grain wall had the same pattern as plants growing *in vivo* and, the aberrant flowers produced at 18°C, as opposed to those produced at 25°C, showed a well-organized tapetum layer during the early phases of microsporogenesis (Santos et al., 2007).

IMPACT OF DROUGHT STRESS ON THE GROWTH OF POLLEN TUBE AND POLLEN GERMINATION

In most plants, pollen germination completely stops under drought. As a result, there is very little research on how dryness affects pollen germination. Fang et al. (2009) found that *in vitro* germination and pollen viability were reduced under drought stress. Compared to well-irrigated plants produced from pollen of irrigated plants, well-irrigated plants formed from pollen pollination of water-stressed plants have fewer germinated pollen grains and fewer pollen tubes reaching the ovary. The decreased germination in both settings implies that pollen tube growth is adversely affected by water deficits applied during flowering. In both cultivated and non-cultivated rice genotypes, decreased pollen viability under water stress results in inadequate fertilization and low spikelet output (Saragih et al., 2013).

CONCLUSION

Abiotic stresses are factors that affect the crops negatively and reduce their yield. Plants can change how they grow and look when they face dryness and high temperatures. These changes are different for different plants. - Plants can grow and develop poorly when there is not enough water and when it is very hot. - The most important part of the plant that can be hurt by drought and heat stress is the part that makes seeds and fruits. The crop yield can be greatly reduced if the plant is stressed at the time when it starts to flower or when it has a lot of grains. These stresses can also harm the parts of the plant that make food, cause harmful chemicals to build up, and make the plant's outer layer weak. Different kinds of plants can handle these problems in different ways. - We have made some progress in reducing the harm caused by these non-living factors on plants. We can do this by changing the plant's genes or by making the plant stronger against the stress. The plants that have been changed to resist heat and drought still have some problems. - Most of the plants that have genes from other plants are not checked in real farms; so, we do not know how well they work in real farms. We can make plants that can grow well in different kinds of stress by using special genes that turn on or off at certain times and places. This way, we can avoid the problem of the genes not working well in different conditions and losing a lot of food.

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Chapter 5

THE EFFECT OF LIGHT SPECTRUM ON PLANT SECONDARY METABOLITES: BIOCHEMICAL STEERING, MOLECULAR MECHANISMS, AND CURRENT APPROACHES

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1. INTRODUCTION

Controlled Environment Agriculture (CEA) and indoor vertical farming systems (plant factories) have undergone a radical technological evolution over the past two decades to break the climate and geographic dependency of the global food supply chain (Kozai and Niu, 2020). In the early generations of these systems, the primary research focus was maximizing the biomass (yield) obtained per unit area by optimizing (temperature, humidity, CO_2, and nutrient solution) environmental parameters (Sambo et al., 2024). High-Pressure Sodium (HPS) lamps and fluorescent lighting, traditionally used in greenhouses and early indoor systems, remained strictly "growth-oriented" and focused on maintaining photosynthesis due to their broad and uncontrollable spectral compositions (Wheeler et al., 1991).

Today, however, driven by shifting consumer demands, the rise in chronic diseases, and the exponential growth in the need for nutraceutical (nutritional and pharmaceutical) foods, the focus in agricultural production has shifted from "quantity" to "quality" (Bian et al., 2015). At the epicenter of this paradigm shift are the revolutionary advancements in narrow-band Light Emitting Diode (LED) technologies and the emergence of the biochemical steering (crop steering) concept in plant science (Folta, 2008).

In traditional agriculture, sunlight is a primary energy source with a spectral composition that cannot be instantaneously controlled. In closed systems, however, LED-based artificial lighting not only provides the Photosynthetically Active Radiation (PAR, 400-700 nm) necessary to drive photosynthesis, but it also acts as a potent and manipulable environmental signal (elicitor) perceived through the plant's photoreceptors (Ouzounis et al., 2015; Kochetova et al., 2022; Paradiso and Proietti, 2022). The qualitative wavelength of photons, their intensity in micromoles (PPFD), and the photoperiod duration directly activate transcription factors in the cell nucleus, fundamentally reprogramming plant morphology, cell wall structure, and secondary metabolite synthesis (Liu et al., 2023).

Secondary metabolites (phenolic compounds, flavonoids, anthocyanins, terpenoids, volatile oils, and glucosinolates) are cellular defense compounds that do not directly participate in the plant's basic growth and reproductive functions (primary metabolism) but are developed against abiotic and biotic stress factors (drought, intense UV radiation, pathogen infections, herbivore attacks) (Verma and Shukla, 2015; Pott et al., 2019). Current literature demonstrates that "controlled abiotic stress" (eustress) induced via artificial lighting systems dramatically increases the accumulation of these protective compounds. However, while the effects of the spectrum on photosynthetic efficiency have been widely elucidated, how specific wavelengths (especially narrow-band Blue/Red ratios and controlled UV-B integration) trigger

enzymatic cascades and gene expressions (mRNA transcript levels) at the cellular level remains an active and profound area of research (Taulavuori et al., 2017; Rai et al., 2021).

The objective of this review is to critically evaluate the effects of different light spectrums applied in CEA on plant photomorphogenesis and secondary metabolite production, detailing them at the transcriptomic and enzymatic levels. In addition to examining how light wavelengths modulate specific biochemical pathways, this study aims to discuss photoperiodic manipulations applied in modern vertical farms, nutrient uptake dynamics linked to stomatal conductance, the effects of pulsed LED frequencies on retrograde signaling, and specifically, the synergistic interactions of innovative nano-elicitors, such as Carbon Nanotubes (CNTs), with light.

2. MOLECULAR DYNAMICS OF PHOTOMORPHOGENESIS AND LIGHT PERCEPTION MECHANISMS

Being sessile organisms, plants possess a highly sophisticated sensory network to survive, compete, and adapt to stress. The process in which specific wavelengths in the light spectrum are perceived at the cellular level, consequently altering the genetic program to trigger physiological and biochemical responses, is called photomorphogenesis (Paradiso and Proietti, 2022: Chang et al., 2025). Plants perceive these photons through specialized light receptors (photoreceptors) structured as chromoproteins and transmit them to the nucleus via complex signal transduction pathways.

2.1. Divergent Roles of Phytochrome A and B, and PIF Regulation

Phytochromes are a family of photoreceptors that mainly perceive the Red and Far-Red light spectrum (Batschauer, 1998). Multiple forms of phytochrome exist with, in some circumstances, identifiably different spectrophotometric, biochemical and physiological characteristics. In the model species Arabidopsis thaliana, five genes (PHYA–E) encoding phytochrome apoproteins have been sequenced and characterized (Li et al., 2011).

· **Phytochrome B (PHYB):** Active under high R:FR (Red:Far-Red) ratios. The inactive Pr exposed to Red light converts into the active Pfr form and migrates to the nucleus. In the nucleus, it binds to negative regulatory proteins called PIF (Phytochrome Interacting Factors)-which promote stem elongation and suppress secondary metabolism-leading to their degradation in the 26S proteasome via ubiquitination (Smith, 2000). The inhibition of PIFs removes the barrier to the transcription of PAL (Phenylalanine ammonia-lyase), the initial enzyme of the phenylpropanoid pathway. The activation of photomorphogenesis in response to light is critically dependent on the repression of several PHYTOCHROME INTERACTING FACTORs

(PIFs), which are basic helix-loop-helix transcription factors that physically interact with phytochromes (Leivar and Monte, 2014). A total of eight PIFs have been identified in *Arabidopsis*, most of which function as repressors of photomorphogenesis (Kunta et al., 2025). Genetic studies have highlighted the role of PIF3 and PIF4 in seedling development, PIF1 in the regulation of seed germination, and PIF7 in promoting shade avoidance responses (Zhang et al., 2019; Zhou et al., 2024).

· **Phytochrome A (PHYA):** Phytochrome A (PHYA), it is active under very low light intensities and Far-Red (FR) light. It is specifically involved in sensing the "Shade Avoidance Syndrome" (Wang et al, 2020). Lowering the R:FR ratio causes the plant to divert energy from secondary metabolite synthesis (defense) to cell wall loosening and internode elongation. Therefore, manipulating the Red and Far-Red balance is the key to carbon partitioning in the cultivation of value-added crops (Maino et al., 2026).

2.2. Cryptochromes, Phototropins, and the Inhibition of the COP1/SPA Complex

Blue (400-500 nm) and UV-A radiation are perceived by flavin-based photoreceptors called Cryptochromes (CRY1, CRY2) and Phototropins (PHOT1, PHOT2) (Christie, 2007; Ponnu, 2020).

· **Phototropins:** Rather than biochemical synthesis, they manage the physical/mechanical adaptations of the plant. Chloroplast avoidance/accumulation movements, phototropism, and most importantly, stomatal opening are governed by phototropins.

· **Cryptochromes:** These are the primary receptors that alter gene expression in biochemical steering. The exponential effect of Blue light on secondary metabolism occurs through the stabilization of a key bZIP (basic leucine zipper) class transcription factor called HY5 (Elongated Hypocotyl 5).

In the dark or environments devoid of Blue light, a massive E3 ubiquitin ligase complex consisting of COP1 (Constitutive Photomorphogenic 1) and SPA proteins continuously degrades the HY5 factor. However, when the plant is exposed to Blue light, the light-absorbing cryptochromes undergo a conformational change, enter the nucleus, and bind directly to the SPA1 protein. This binding dissociates and inactivates the COP1-SPA complex (Mishra and Khurana, 2017). HY5, rescued from degradation and rapidly accumulating in the nucleoplasm, binds directly to specific light-responsive elements (LREs) in the promoter regions of genes responsible for the biosynthesis of volatile oils, anthocyanins, and glucosinolates, up-regulating them (Maino et al., 2026).

2.3. UVR8 Receptors and the UV-B Induced Eustress (Hormesis) Mechanism

While Ultraviolet-B (UV-B, 280-315 nm) is a destructive radiation type causing DNA lesions and protein denaturation at high doses, it acts as a potent eustress source that triggers the plant's defense mechanisms when applied in controlled, low doses (Hideg et al., 2013). This dose-response relationship is termed Hormesis in the literature.

UV-B photons are perceived by a unique receptor called UVR8. Unlike phytochromes and cryptochromes, UVR8 does not use a chromophore. In instead, it directly absorbs UV-B photons via specific tryptophan amino acid residues (Trp285 and Trp233) in its protein sequence. Upon UV-B exposure, the homodimeric structure dissociates into monomers, and similar to cryptochromes, UVR8 binds to COP1, sequestering it and ensuring HY5 accumulation (Heijde and Ulm, 2012; Ponnu, 2020; Zhan and Zhu, 2025). This unique mechanism commands the massive synthesis of flavonoids (quercetin and kaempferol glycosides) in the epidermal layers to act as a biological sunscreen against potential solar damage. Simultaneously, it maximizes the production of phytoalexins-used by the plant against biotic stresses (fungal pathogens)-and the activities of antioxidant enzymes (Superoxide dismutase, Catalase) at the cellular level.

3. TRANSCRIPTOMIC EFFECTS OF LIGHT SPECTRUM ON SPECIFIC SECONDARY METABOLITE PATHWAYS

The narrow-band spectral control capability offered by LED technologies in CEA systems provides the opportunity to "hack" the Shikimate, Phenylpropanoid, and Terpenoid biosynthesis pathways, going far beyond primary metabolism. This capacity is examined below at the cellular and genetic levels through specific model plants with high economic and pharmacological value.

3.1. The Glucosinolate-Myrosinase System and Sulfur Assimilation: The Watercress (*Nasturtium officinale*) Model

Watercress (*Nasturtium officinale*) stands at the pinnacle of pharmacological agriculture due to its high adaptability to hydroponic systems and its unique sulfur-containing compounds. Gluconasturtiin, a type of glucosinolate hydrolyzed by the myrosinase enzyme stored in vacuoles upon tissue damage, converts into Phenethyl Isothiocyanate (PEITC), one of the most potent anticancer (carcinogenesis and angiogenesis inhibitor) molecules known in the literature (Kyriakou et al., 2022; Kattel and Antonious, 2025)

The manipulation of glucosinolate biosynthesis by light spectrum in watercress occurs through the responses of *MYB28, MYB29* (for aliphatic glucosinolates) and *MYB34, MYB51* (for indolic glucosinolates) transcription factors to light at the cellular level. Controlled environment experiments demonstrate that increasing the Blue (400-500 nm) light ratio to the 30-40%

band in artificial lighting recipes severely up-regulates the expression of the *CYP79* and *CYP83* cytochrome P450 gene families, which catalyze the first step in glucosinolate synthesis (Yatusevich, 2008).

Furthermore, the root uptake and assimilation of sulfur (S), the fundamental building block of glucosinolates, is indirectly accelerated as Blue light increases stomatal conductance (transpiration pull). The synergistic use of Blue and Far-Red light (under high PPFD) not only preserves cellular ascorbic acid synthesis in watercress but also increases gluconasturtiin content by 55% compared to standard greenhouse conditions (or Red light alone), transforming watercress into a standardized pharmacological raw material (Lam et al., 2021).

3.2. The MBW Complex and Anthocyanin Biosynthesis Cascades: The Red Leaf Lettuce (*Lactuca sativa*) Model

This is a model representing the physiological and metabolic responses of red-leaf lettuce under different lighting conditions. In Red Leaf Lettuce (Lactuca sativa var. crispa), anthocyanin synthesis depends on a complex enzyme cascade located downstream of the phenylpropanoid pathway (Zhou et al., 2025).

The "orchestrator" of this long enzymatic cascade in the nucleus is the structure termed the MBW complex (a triad of MYB-bHLH-WD40 transcription factors). The *PAP1/MYB75* factor within the MBW complex is directly activated by the Cryptochrome 1 (CRY1) photoreceptor and HY5. In traditional greenhouses, lettuces that are genetically "red/purple" often remain green and lose their market value under low light intensity or incorrect spectrum. However, in CEA systems, increasing Blue light above 50% or integrating UV-A radiation hyper-activates the MBW complex, exponentially increasing CHS and DFR transcript levels in as little as 48 hours. This rapid reaction is the greatest evidence of how sensitive secondary metabolites are to the spectral "switch" mechanism (Zhang et al., 2018).

3.3. Compartmentalization of MEP/MVA Pathways and Chemotype Manipulation: The Peppermint (*Mentha x piperita*) Model

Peppermint (*Mentha x piperita*) is an irreplaceable model plant for the pharmaceutical and cosmetic industries due to its monoterpenes (menthol, menthone, pulegone, cineole). The characteristic scent profile of peppermint is synthesized and stored in specialized peltate glandular trichomes on the leaf epidermis.

At the molecular level, terpenoid biosynthesis is subject to strict compartmentalization within the plant cell. The synthesis proceeds via Geranyl Pyrophosphate (GPP), the common product of the Mevalonate pathway occurring in the cytosol and the Methylerythritol Phosphate pathway

occurring in the chloroplasts/plastids (Sabzalian et al., 2014). Light spectrum directly affects the MEP pathway in plastids. An increased Blue light ratio not only increases trichome density in peppermint but also accelerates the conversion of pulegone-a toxic precursor in the monoterpene pathway-into the target compound menthol via pulegone reductase and menthone reductase enzymes.

Conversely, under conditions where Red light (660 nm) is highly dominant, menthol conversion slows down, leading to the accumulation of industrially undesirable pulegone/menthofuran in the plant. This cellular steering capability demonstrates that artificial lighting fundamentally reprograms not just biomass, but the "chemotype" (chemical sub-species) that determines the pharmacopoeia standards of the product via light quality (Behn et al., 2010).

4. CIRCADIAN CLOCK, PHOTOPERIOD, AND ASSIMILATION DYNAMICS

Just as the spectral quality (wavelength) and intensity (PPFD) of light are critical, the duration of its application (photoperiod) and timing exert a decisive influence on secondary metabolism. The absence of seasonal photoperiod restrictions (the necessity of sunset) in CEA systems has led researchers to develop innovative regimes that challenge the plants' circadian clock.

4.1. End-of-Day (EOD) Lighting

Plants perceive the light spectrum during the final hours of the day as a "timing and seasonal signal." Traditionally, the Red:Far-Red (R:FR) ratio in sunlight drops at sunset due to atmospheric scattering. In vertical farms, applying a brief (15-30 minutes) EOD Far-Red (EOD-FR) illumination after the main lighting cycle ends rapidly converts all phytochromes from the Pfr form to the inactive Pr form. This intervention regulates flowering signals (florigen production) as the plant enters the dark period and increases the translocation of photo-assimilates (carbohydrates) to generative "sink" organs such as fruits and seeds rather than roots and stems (Kong et al., 2025). Similarly, EOD Blue (EOD-B) applications have been reported to delay stomatal closure, sustaining nocturnal transpiration and enhancing cellular robustness by increasing the transport of immobile elements like calcium into tissues.

4.2. Continuous Lighting (CL) and the Modulation of Circadian Clock Genes

Plants require a dark period to transport carbohydrates (starch) to roots and respiratory organs. The application of 20 to 24 hours of Continuous Lighting (CL) leads to excessive carbohydrate accumulation (hyper-

accumulation) in the leaves, ultimately causing "photosynthetic feedback inhibition" and placing the plant under severe biochemical stress (Sysoeva et al., 2010). However, from the perspective of biochemical steering, CL application presents a tremendous opportunity for secondary metabolite synthesis. The accumulation of carbohydrates in the leaf creates an inexhaustible "carbon skeleton" pool for secondary metabolite (especially phenolic acid) synthesis. In medicinal plants grown under 24-hour continuous LED lighting, the expression profile of key genes governing the circadian clock (*LHY, CCA1, TOC1*) is disrupted/modified, keeping defense mechanisms in a continuous "on" state (Velez-Ramirez et al., 2011).

5. LIGHT AND RHIZOSPHERE INTERACTIONS: STOMATAL CONDUCTANCE AND NUTRIENT UPTAKE

Biochemical steering reaches a systemic dimension not merely by photons hitting the leaf, but by light responses in the leaf influencing nutrient and water uptake dynamics in the root zone (rhizosphere).

The opening and closing of stomata on plant leaves are regulated by Blue light-sensitive phototropin receptors in the guard cells. Blue light activates phototropins, driving plasma membrane H+-ATPase pumps in the guard cells. Protons (H+) pumped out of the cell open potassium (K+) channels, allowing water influx into the cell, which opens the stomata to full capacity.

Increasing the Blue light ratio in CEA maximizes stomatal conductance and, consequently, transpiration pull. Increased transpiration allows the roots in the hydroponic system to draw significantly more water, Nitrogen (N), and Sulfur (S) from the solution. Sulfur is the building block of the glucosinolates in watercress and is also a componant of many antioxidant enzymes (Lam et al., 2021). Wavelength and light quality, particularly the presence of Blue light, affects not only the plant's enzymatic processes but also the uptake and transportation of mineral nutrients from the root to the leaf.

6. BIOCHEMICAL STEERING (CROP STEERING) STRATEGIES

6.1. End-of-Production (EOP) Lighting and Reactive Oxygen Species (ROS) Signaling

There is an evolutionary energy-sharing dilemma (trade-off) between carbon assimilation (growth) and secondary metabolite synthesis (defense) in plants (Herms and Mattson, 1992). In the EOP strategy, the plant is grown under growth-oriented (High Red/Low Blue) light for the first 80% of the cycle. Just 3 to 7 days before harvest, the spectrum is abruptly switched to 100% Blue or UV-A (Carvalho and Folta, 2014).

This acute photon stress strains the photosystems in the chloroplasts, causing an instantaneous burst of Reactive Oxygen Species (ROS - Hydrogen

peroxide, superoxide radicals). ROS molecules act as secondary messengers within the cell, stimulating the retrograde signaling pathway between the mitochondria and the nucleus. Without the plant experiencing biomass loss (because harvest is days away), the phenylpropanoid pathway activated by ROS signals produces massive amounts of antioxidants.

6.2. Pulsed LED Lighting and Chloroplast Redox Fluctuations

Pulsed (Flickering) Lighting, applied by switching LED chips on and off hundreds or thousands of times per second (microsecond-level duty cycle manipulation), is a relatively recent revolution for plant physiology (Lefsrud et al., 2006). Pulsed lighting synchronizes the speed mismatch between the light-dependent reactions of photosynthesis (ATP/NADPH production) and the dark reactions (Calvin cycle carbon fixation).

Pulsed Blue light at specific frequencies continuously alters the redox state of the plastoquinone pool in chloroplasts within milliseconds. These rapid oxidation-reduction fluctuations optimize the Non-Photochemical Quenching heat dissipation mechanism and initiate powerful retrograde signaling from the chloroplast to the cell nucleus (Wilson and Ruban, 2020). Consequently, secondary metabolite genes that remain inactive under continuous lighting are triggered.

7. FUTURE PERSPECTIVES AND PLANT NANOBIONICS (RESEARCH GAPS)

Although the integration of LED technologies into CEA has rewritten photobiology, massive and multi-layered research gaps exist in the academic literature. The most critical perspective that will guide future doctoral studies is the convergence of light with nanomaterials.

7.1. Nano-Elicitors and Spectral Steering Synergy (The Plant Nanobionic Model)

The most profound research gap in current literature is the synergistic molecular cascades created by specific narrow-band light spectrums combined with innovative nanomaterials applied to the cultivation solution. In particular, the integration of multi-walled carbon nanotube and single-walled carbon nanotube into hydroponic systems has forged a completely new frontier in the field of Plant Nanobionics (Giraldo et al., 2014).

Due to their nanoscale dimensions, high specific surface areas, and superior electrical/semiconductor properties, carbon nanotubes easily penetrate the plant root epidermis, entering the vascular tissue (xylem). Reaching the leaves via the transpiration stream, CNTs possess the capacity to cross the double-layered lipid membranes of chloroplasts and embed themselves into the thylakoid lumen.

In visionary projections modeling value-added crops with high phenolic and volatile oil potential, such as Sweet Basil (*Ocimum basilicum*), the intersection of Carbon Nanotube integration with the light spectrum (Blue light or UV stress) promises a colossal biochemical synergy. This nanobionic fortification manages the chloroplast stress and ROS production that occurs when the plant is subjected to narrow-band photon bombardment (e.g., 100% Blue Light EOP application), acting similar to an "electron sponge and conductor." At the cellular level, the amalgamation of the systemic "nano-stress/elicitation" signal generated by root-absorbed CNTs with the Blue light (Cryptochrome/HY5 pathway) striking the leaf has the potential to exponentially upregulate the expression of PAL (Phenylalanine ammonia-lyase), the key enzyme of the phenylpropanoid pathway (Gohari et al., 2020). Future research should map via transcriptomic sequencing (RNA-Seq) how CNT concentrations, supported by specific light spectrums, can push rosmarinic acid and eugenol production beyond traditional genetic yield limits in species like basil. This unique interaction of light and carbon-based nanomaterials is the high-impact research area that will define the future of pharmaceutical agriculture.

7.2. Autonomous AI Light Recipes and Dynamic Optimization

The vertical farms of the future will rely on Artificial Intelligence (AI) supported closed-loop autonomous systems that detect the real-time biochemical stress status of the plant second by second using multispectral cameras (chlorophyll fluorescence, thermal, and hyperspectral sensors) (Wang et al., 2024). Models that detect microscopic increases in leaf surface temperature (stomatal closure) or the first cellular reflections of anthocyanin synthesis, and instantaneously optimize spectrum ratios using "Reinforcement Learning" algorithms, represent the next engineering leap in agronomic research.

8. CONCLUSION

Controlled Environment Agriculture has evolved from a quantitative discipline focused solely on biomass production into a qualitative "bio-manufacturing" process that optimizes plant genetics, circadian rhythms, and enzymatic cascades at the molecular level. At the heart of this transformation lie narrow-band LED technologies and biochemical steering (crop steering) strategies that program nuclear gene expression by manipulating plant photoreceptors.

The transcriptomic mappings examined within the scope of this review have proven that photon quality is the most fundamental key governing plant defense mechanisms. The stimulation of isothiocyanate (PEITC) conversion in watercress by the Blue/Far-Red spectrum and sulfur assimilation fueled by stomatal pull; the hyper-activation of the MBW complex by EOP-induced ROS

signals in red leaf lettuce; and the rewriting of the chemotype in peppermint by light-modulating the MEP pathway demonstrate that light is not merely an energy source, but a massive code sequence. Furthermore, photoperiodic forcings (Continuous Lighting - CL) and Pulsed light frequencies possess the potential to optimize the secondary metabolism pool and energy costs in unprecedented ways.

In a future perspective, photobiological optimizations in modern plant factories must integrate with nanobionic synergies created by the tissue penetration of nano-elicitors like Carbon Nanotubes, and closed-loop systems autonomously managed by machine learning (AI). The convergence of cellular photon targeting with the eliciting power of nanomaterials will not only flawlessly build the production of nutritionally enhanced foods but also the industrial "pharma-farming" vision of the future.

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Chapter 6

BIOLOGICALLY ACTIVE COMPOUNDS (SECONDARY METABOLITES) IN MYXOGASTRIA II: AMINO ACIDS, PEPTIDES AND PROTEINS

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Introduction:

Primary metabolites are produced in connection with the growth, development and maturation phases of the organism, while the production of secondary metabolites occurs only after active growth and development, in the stationary phase. Changes in growth conditions, sudden changes in environmental factors, can alter the timing of the initiation of secondary metabolism and secondary metabolites can be produced simultaneously with growth (Aharonowitz and Demain, 1980). The function of secondary metabolism is to ensure that metabolism remains functional in situations where growth is not possible; it "may function in controlling the orderly sequence of events occurring during differentiation" (Weinberg, 1971). Furthermore, there have been many proponents of the view that secondary metabolites may provide an ecological advantage in the organism (Brian, 1957; Bakır, 2020).

There are many relationships between morphogenesis and secondary metabolism. In general, both processes begin after active growth has ceased. Both processes tend to have requirements for trace amounts of metals, pH, and temperature, but within narrower ranges compared to growth (Bennett, 1981).

Biologically Active Compounds are uses in people medicine, veterinary medicine, plant, scientific research, and countless other fields. Secondary metabolites is important in the cancer, pharmaceuticals, malaria, bacteri and fungi infections, neurological and cardiovascular diseases, and autoimmune disorders. Antibiotics were formerly defined as secondary metabolites isolated from microorganisms exhibiting antimicrobial, antitumor, and/or antiviral activity. Today the original definition should be broadened to include all Biologically Active Compounds that, at a minimum concentration at the biochemical level, regulate and/or trigger a type of response (regulatory, inhibitory, stimulatory) to the growth processes and reproduction of prokaryotic organisms or eukaryotic cells (Berdy, 2005; Baba and Akgül, 2025a).

In summary, most known non-antibiotic biological activities can be classified as follows:

– Those exhibiting pharmacological, biochemical and medicinal properties,

– Those exhibiting agricultural,

– Those exhibiting regulatory, biophysical and other.

Secondary metabolites can be produced both prokaryotic and eukaryotic organisms. They can be produced in varying amounts and types by plants,

animals, fungi, protists, and organisms belonging to the kingdom Monera. However, the ability to produce secondary metabolites is quite uneven among organisms (Berdy, 2005).

Since ancient times humans have primarily obtained their basic metabolite needs, such as carbohydrates, proteins, and fats from plants. In addition to these essential metabolites, which are of great importance to humans, plants also contain secondary metabolites. These serve various functions within the plant, including transport, energy, growth and differentiation, social communication, defense, reproduction, dispersal, ecological adaptation, environmental adjustment, resistance to environmental stressors such as drought, salinity and UV, protection against herbivores and microorganisms and facilitating pollination and seed dispersal. These metabolites are consumed by humans and used in food, cosmetics, agriculture and medicine. These compounds also include plant metabolites (alkaloids, β-avonoids, terpenoids, etc.) that possess antimicrobial, antitumor, antibiotic, toxic, and pharmacological effects. In the broadest sense, all the compounds mentioned above can also be considered antibiotics (Gilbert and Raven, 1975; Swain, 1977; Rosenthal and Janzen, 1979; Vuran and Türker, 2021).

Fungi are a rich source of active secondary metabolites that make significant contributions to human and animal health. Secondary metabolites in fungi can include simple molecules directly derived from primary metabolism. Among the best-known examples are beta-lactam antibiotics, including penicillins and cephalosporins. These classes of compounds have made crucial contributions to global health due to their efficacy against bacterial infections (Akata et al., 2025; Baba et al., 2025).

Secondary metabolites in animals are organic compounds that are generally synthesized in specialized cells and at specific developmental stages. While these metabolites are not directly necessary for the growth, development, or reproduction of animals, they play important roles in various areas such as defense, communication, reproduction, and competition. For example, toxins found in some marine organisms play a role in protection from predators, while pheromones in insects are critically important for intraspecific communication. Additionally, secondary metabolites in some animals can also play a role in functions such as pigmentation, antimicrobial activity, and UV protection. The diversity and complexity of these compounds offer an important adaptation mechanism in animals' interactions with their environment. In recent years, marine soft-bodied invertebrates (sponges, mollusks, cnidarians, tunicates, echinoderms, etc.) in the Animal Kingdom have proven to be rich sources of various bioactive metabolites (Avhad and Bhangale, 2023).

In prokaryotic organisms, Bacillus and Pseudomonas species are rich producers of secondary metabolites. The total number of known bioactive compounds in this group is approximately 3800, constituting 17% of all microbial metabolites. Filamentous Actinomycetales species produce more than 10,000 bioactive compounds; 7600 of these are derived from Streptomyces and 2500 from rare actinomycetes, representing the largest group (45%) of bioactive microbial metabolites. Clearly, various Actinomycetes, Streptomyces species and filamentous fungi and to a lesser extent various bacterial species, are the most remarkable producers in terms of both their numbers and versatility, and the structural diversity of the metabolites produced. In recent years, interest in bacteria has increased, and the proportion of naturally occurring actinomycete compounds has decreased (Bills and Gloer, 2016).

In addition to the microorganisms mentioned above, algae, dinozagellates, and diatoms are relatively frequent producers, generating over 1300 bioactive metabolites. Lichens and Bryophyta species also produce hundreds of antibiotic-active metabolites. Lichens are complex symbiotic associations between fungi and algae, which are important components of many ecosystems. To date, more than 800 lichen secondary metabolites have been discovered, many of which are found only in lichens. Lichens and their secondary metabolites have numerous pharmaceutical roles, primarily exhibiting antimicrobial, antioxidant, antiviral, anticancer, antigenotoxic, anti-inflammatory, analgesic, and antipyretic activities (Rankovic and Kosanic, 2015).

Among fungal species, certain microscopic fungi within Ascomycetes and Fungi imperfecti are among the most frequent producers of secondary metabolites, with approximately 6400 compounds identified. Secondary compounds have been isolated especially Ascomycetes species such as Aspergillus, Penicillium, and Fusarium. Additionally, several other reticulate and endophytic species are also good producers. From higher fungal species such as Ganoderma and Lactarius (Baidiomycetes), a total of approximately 2000 active compounds have been obtained. Only 140 biologically active metabolites have been isolated from yeasts, and 60 from myxomycetes (slime molds). Chemically relatively simple, fungal compounds exhibit a variety of biological effects, including antibiotic activity and, particularly, phytotoxic and pharmacological effects. However, we know that beta-lactams such as penicillins and cephalosporins, cyclosporines, and statins such as mevinolin, compactin, lovastatin, pravastatin, and atrovastatin are all fungal-derived compounds. We must not forget that these secondary metabolites have great practical importance (Berdy, 2005; Çapar et al., 2025).

Eumycetozoa, also known as slime molds, form a monophyletic clade within the phylum Amoebozoa, which includes Myxogastrids, Dictyostelids and Protostelids (Lado, 2005-2026). Members of this group are very small,

making it nearly impossible to collect many species directly in the field. Instead, research on slime molds is conducted in the laboratory. For moist chamber culturing various plant materials brought from the field. Slime molds feed on yeasts, bacteria, protozoa, and other small organisms on decaying organic matter and ingesting them (Stephenson and Stempen, 1994).

Although myxogastrids were historically misclassified as fungi for a long time, they are now grouped in the kingdom Protista. Recent studies have shown that these organisms produce a wide variety of bioactive secondary metabolites. Myxomycetes are mostly terrestrial organisms and thrive in environments rich in decaying or rotting organic plant matter. They feed on various microorganisms on decaying plant debris such as leaves, wood, and cones, as well as on herbivorous animal manure. Myxomycetes are characterized by their true plasmodium and often prominent fructification. Most Myxomycetes are microscopic but some members produce visible vegetative and generatif structure (Baba and Sevindik, 2018).

Because myxogastria has a complex and multi-stage life cycle, it contains different bioactive compounds, such as plasmodium, sporophores, myxamoeba or myxophilacellate, spores, and sclerotium. Myxogastria exhibit striking diversity in fruiting body sizes and colors, ranging from 50-500 μm. This extreme morphological diversity makes it difficult to collect sufficient biomass for chemical analysis, but it is also known to possess a potentially rich bioactive compound content (Sevindik et al., 2018).

Approximately 1200 species known so far, very few have been investigated. Laboratory culture of myxomycetes appears to be an effective way to culture plasmodials and obtain sufficient metabolites. Current laboratory culture methods mainly include moist chamber culture, feed culture, pure culture, liquid culture and hanging drop culture. Among these, moist chamber culture is the most common culture method (Li et al., 2020).

Materials and Methods:

The life cycle of Myxogastria consists of two main stages: plasmodium and fruiting bodys. These stages represent the vegetative and generative phases. Mature spores germinate under suitable temperature and humidity conditions, producing one or more (usually four) myxoamoeba or myxoflagellate. The type and development of these cells vary depending on the amount of water and moisture in the environment. Myxoamoeba or myxoflagellate feed, grow, and divide, ultimately forming a distinct population of numerous cells. In unfavorable environmental conditions, these cells continue to survive by forming durable structures called microcysts for self-protection. Myxoamoeba or myxoflagellate structures reappear when suitable conditions are provided, forming independently of cell shape. The cells fuse at their posterior ends to form diploid cells, or zygotes. Zygotes undergo synchronized and rhythmic

mitotic divisions in their nuclei and, while maintaining nutrient uptake, develop into plasmodium, a multinuclear but acellular structure. However, when environmental conditions become unfavorable, plasmodiums transform into durable structures called sclerotium. When suitable conditions are restored, the sclerotium transforms back into plasmodium and continues development. These plasmodiums, if environmental conditions permit, form sporophore structures characteristic of their species. A mature plasmodium may develop one or more sporophores specific to its species (Baba and Akgül, 2025b).

Biochemical studies on microbial secondary metabolites of myxomycetes being conducted on plasmodium, sclerotium, and fruiting bodies. If plasmodium, sclerotium and fruiting bodies of species could be examined, it would be possible to obtain many more primary and secondary metabolites (Baba et al., 2020).

Dembitsky et al., (2005) reported more than 100 secondary metabolites. According to Pawłowicz et al. (2025), have been organized into 8 main categories. Recent molecular and biochemical studies have revealed the rich metabolomic profiles of slime molds, identifying 298 different metabolites.

Results:

Amino Acids, Peptides, and Proteins: in Eumycetozoa are studied by dividing them into 2 groups: Proteinogenic and non-proteinogenic amino acids and enzymes, peptides and macromolecular proteins (Pawłowicz et al., 2025).

a. Proteinogenic and Non-Proteinogenic Amino Acids: Proteinogenic amino acids are those commonly found in proteins. Non-proteinogenic amino acids can be post-translationally modified residues or entirely distinct structures with specialized functions. Amino acids, peptides, and proteins are crucial in the realization of vital physiological processes in true slime molds. They contribute to essential metabolic pathways, form actomyosin, and are precursor or regulatory molecules for many others. The amino acid composition of plasmodial slime molds controls changes associated with spore formation, spore germination, and microcyst formation and development. Pawłowicz et al., (2025) reported 16 amino acids in spores plasmodia and microcysts of Physarum polycephalum. Sonobe et al., (1994) reported 18 amino acids obtained from P. polycephalum myxoamoebas. Kuehn et al., (1979) reported 17 amino acids. An acidic nucleolar phosphoprotein was purified from isolated nuclei of Physarum polycephalum. The amino acid composition of this phosphoprotein is similar to that of a nucleolar phosphoprotein obtained from acidic cells of Novikoff hepatoma.

b. Enzymes, Peptides, and Macromolecular Proteins: Proteins play a role in biological catalysis (enzymes), cellular cytoskeleton, and cell adhesion processes in Myxogastria. They are involved in cellular metabolism, development, morphogenesis, and adaptation to environmental interactions (Pawłowicz et al., 2025).

Significant amounts of haemagglutinins were found in the mucus layer on the surface of the plasmodium of Physarn polycephalum. However, an acceleration of hemagglutinin synthesis was observed during globularization and fruiting body formation. Soluble hemagglutinins, designated haemagglutinin I and II, with molecular masses of 6 and 11 kDa, respectively, were isolated from the plasmodes of P. polycephalum. P. polycephalum. hemagglutinins are only found in the plasmodial phase (diploid) and are not detected in myxamoebas (haploid) (Morita et al., 1995). Yokota and Nitta (1996) purified a hemagglutinin called pallidin and fizarumin. Glorin, pallidin, and physarminin (hemagglutinin) were detected in different structures (Plasmodium and Fruit Body) obtained from Physarum polycephalum. These protein compounds play a role in different life cycle stages of Myxogastria, from the growth and development of the organism's plasmodium and sporophore formation to the formation and maturation of spores.

HMG-like proteins (High mobility group protein) were isolated from the nuclei of Physarum polycephalum. HMG-like proteins have been also found in mammals, birds insects, yeast as well as in plants. Current data clearly demonstrate that these proteins differ significantly from vertebrate HMG proteins. Physrum polycephalum HMG-like proteins differ from those in animal cells in terms of electrophoretic behavior. They have been found to contain 3 main HMG-type proteins localized within active chromatin. Nuclei were isolated from plasmodia. These proteins are found in the nuclei of Physarum polycephalum in amounts similar to those found in vertebrate animals. Physarum proteins also differ structurally from HMG-like proteins in other eukaryotic organisms. However, their distribution within the chromatin behaves similarly to animal cell HMG proteins. Despite their different molecular properties, they are thought to perform similar functions (Czupryn and Toczko, 1984).

An acidic nucleolar phosphoprotein (Polyamine) was purified from isolated nuclei of Physarum polycephalum. The protein was purified in isolated nuclei after selective phosphorylation via a polyamine-mediated reaction without the use of strong interferences. Its amino acid composition is similar to that of a nucleolar phosphoprotein obtained from acidic cells of Novikoff hepatoma proteins. Thus, a specific phosphoprotein in a eukaryote capable of stimulating the transcription of a particular gene cluster was isolated. These proteins have been found to play a role in controlling and promoting cell growth processes, as well as being associated with the metabolism, structure,

and function of nucleic acids, and having a role in stimulating rRNA synthesis (Kuehn et al., 1979).

In the eukaryotic cell nucleus, the basic structure of chromatin is the nucleosome, formed by DNA coiling twice around a histone core (Wolffe, 1998). Mitochondrial DNA (mtDNA), along with proteins, is packaged in the mitochondrial nucleoid (mt-nucleoid) or nucleus. In some animals, plants, and fungi, mt-nucleoids can often be observed as small dots. Glom is a novel mitochondrial DNA packaging protein in Physarum polycephalum. These proteins are rich in lysine and homologous to histone H1 proteins. Glom is located throughout the entire mitochondrial nucleoid. P. polycephalum has highly condensed, rod-shaped mitochondrial nucleoids. Glom is present in all mitochondrial nucleoids except the nucleus. It is homogeneously distributed throughout the mitochondrial nucleoid (Sasaki et al., 2003).

Mitochondrial DNA (mtDNA) is organized into mitochondrial nucleoids (mt-nucleoids), which are highly ordered structures. As part of studies on the nucleoid structure of mitochondrial DNA, a novel mt-nucleoid protein, Pmn34 (Physarum polycephalum mitochondrial nucleoid protein 34), has been identified from the true mucus fungus Physarum polycephalum. The Pmn34 protein consists of a total of 326 amino acids, including mitochondrial transition peptides. Mitochondria are a common organelle in eukaryotes that contains its own DNA, namely mitochondrial DNA (mtDNA). Physarum polycephalum contains an extraordinary amount of mtDNA in its rod-shaped mitochondrial nucleoids (Kuroiwa, 1982).

SDS-PAGE analysis has shown that the mt-nucleoid isolated from P. polycephalum consists of at least 70 different proteins; among these, Glom was identified as the most abundant protein and functions as a histone-like DNA packaging protein (Sasaki et al. 2003; Itoh et al., 2009).

Glom and Glom2, identified as DNA packaging proteins, progressively regulate the mitochondrial nucleoid of Physarum polycephalum. Mitochondrial DNA is typically packaged into mitochondrial nucleoids by high mobility group (HMG) proteins. Glom is an HMG protein that packages mtDNA in Physarum polycephalum. Here, Glom2, a novel mtDNA packaging protein with a region homologous to yeast Mgm101, is identified. Glom2 has the ability to bind to all mtDNA and works together with Glom for in vitro mtDNA condensation (Itoh et al., 2011).

From the plasmodium, the vegetative stage of the Myxogastria species Physarum melleum, two novel peptide lactones, Melleumin A and Melleumin B, containing acetic acid linked to a tyrosine, an amino acid, have been isolated. Melleumin A consists of four amino acid residues. (Dembitsky et al., 2005; Nakatani et al., 2005a).

3,4-dihydroxyphenylalanine (L-DOPA), a neurotransmitter precursor with antimicrobial and anticancer properties used in the treatment of Parkinson's disease, has been isolated from the sporophore and plasmodium of myxomycetes. The commercialization potential of this compound obtained from Stemonitis herbatica was studied and reported to the scientific community by Loganathan (1998).

Myosin has been isolated from Myxogastria amoebas and plasmodes called Physarum polycephalum. Amoebic myosin is very difficult to distinguish from plasmodial myosin in terms of ATPase activity and molecular shape. However, the heavy chain and phosphorylatable light chains of amoebic myosin could be distinguished from those of plasmodial myosin by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, peptide mapping, and immunological studies. These results suggest that they are different gene products. Regarding whether amoebic and plasmodial myosins are identical, their Ca2+ binding light chains were found to be identical using similar criteria (Kohama et al., 1986).

Myosins are essential proteins for all types of cellular movement activities in myxomycetes. Myosins have been purified and studied in detail in slime molds to investigate cytokinesis and cell movement. There are 35 classes of myosins known to play a role in cell adhesion, endocytosis, and exocytosis (Gärtner et al., 2023).

Conclusions:

In this study, we investigated the protein-structured compounds, molecules, and peptide metabolites produced by myxomycetes. Eighteen of the 20 known essential amino acids in myxomycetes were identified. These protein-based secondary metabolites play crucial roles in the different developmental stages of Myxogastria (plasmodium, nutrition, spore formation, and sporophore formation) and ecological functions (protection, antimicrobial defense, environmental adaptation) of myxomycetes. Providing food and healthcare for billions of people is one of humanity's most urgent needs. Increased food production and consumption, environmental problems, and high economic costs highlight the importance and advantage of secondary metabolites compared to synthetic chemicals. Most environmentally friendly food compounds are natural products or obtained by modifying natural product derivatives. Currently, approximately 60% of known microbial metabolites exhibit antimicrobial (antibacterial, antifungal, antiprotozoal) properties, while about 5,000 compounds demonstrate antitumor activity and approximately 1,500 compounds exhibit antiviral activity. Less than 1% of known antibiotics and microbial compounds, approximately 150 compounds, are directly used in human and veterinary medicine and agriculture. These secondary metabolites have various biological roles (specifically antimicrobial,

cytotoxic, or signaling). Ongoing biochemical, genomic, and ecological research on myxomycetes will lead to the discovery of new secondary metabolites. These newly obtained metabolites will contribute to the well-being of humanity in fields such as medicine, pharmacy, agriculture, food, veterinary medicine, and the environment.

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