

INTEGRATED AGRICULTURAL SYSTEMS AND TECHNOLOGICAL APPROACHES

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BİDGE Yayınları

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Approaches**

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PREFACE

The rapid growth of the global population, the increasingly evident impacts of climate change on agricultural productivity, the growing scarcity of natural resources, and the rising demand for safe and sustainable food production are driving the agricultural sector toward a profound transformation. Today, traditional approaches that focus solely on increasing productivity are no longer sufficient. Instead, holistic production systems that simultaneously address economic viability, environmental stewardship, and social sustainability have become increasingly important. At the center of this transformation is the integration of agricultural production systems with advanced digital and smart farming technologies.

Integrated agricultural systems are based on the principle of managing crop production, livestock, aquaculture, forestry, and other agricultural activities in an integrated and mutually supportive manner. Their primary objectives are to improve resource-use efficiency, enhance nutrient recycling within production systems, reduce production risks, and strengthen the economic resilience of agricultural enterprises. Contemporary research consistently demonstrates that well-designed integrated systems, in which interactions among different production components are effectively managed, can significantly reduce environmental impacts while enhancing the long-term sustainability and resilience of agricultural production.

Recent technological advances have introduced a new dimension to integrated agriculture. Precision agriculture, artificial intelligence-based decision support systems, remote sensing technologies, unmanned aerial vehicles (UAVs), big data analytics, robotics, the Internet of Things (IoT), and geographic information systems (GIS) have substantially improved the monitoring and management of agricultural production. These technologies enable more efficient use of natural resources, optimize production inputs, and support evidence-based decision-making, thereby improving productivity while reducing production costs and minimizing environmental impacts.

This book aims to provide a comprehensive overview of integrated agricultural systems and the technological innovations that are reshaping modern agriculture. The chapters examine key topics including sustainable agricultural practices, climate change adaptation strategies, precision agriculture, smart sensor technologies, data-driven farm management, digital agriculture, natural resource management, and innovative production systems. Each chapter combines current scientific knowledge with practical applications to provide readers with both theoretical foundations and real-world perspectives.

Adopting a multidisciplinary approach, this volume brings together expertise from agricultural engineering, crop and soil sciences, animal science, environmental science, information technologies, and sustainable development. By integrating knowledge across these disciplines, the book seeks to promote a comprehensive understanding of modern agricultural systems and encourage innovative solutions to current and future agricultural challenges. It is our hope that readers will not only gain insight into existing technologies but also develop a broader perspective on the emerging innovations that will shape the future of global agriculture.

This book is intended to serve as a valuable reference for undergraduate and graduate students, researchers, academics, agricultural consultants, policymakers, extension specialists, and professionals working throughout the agricultural sector. We also hope that it will contribute to the dissemination of scientific knowledge and support the development of sustainable agricultural practices and policies at both regional and global levels.

The future of agriculture will be built upon production systems that harmonize environmental sustainability with technological innovation, promote efficient resource management through data-driven approaches, and strengthen resilience in the face of global challenges. It is our sincere hope that this volume will contribute to the advancement of scientific knowledge, inspire future research, and support the transition toward more sustainable, resilient, and technology-driven agricultural systems.

Finally, we would like to express our sincere gratitude to all contributing authors for their valuable expertise, dedication, and commitment throughout the preparation of this book. Their scientific contributions have made this volume possible, and we deeply appreciate their efforts.

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2026

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MEHMET AKYOL

CHAPTER 1

ADVANCES AGRICULTURE OF ARTIFICIAL INTELLIGENCE

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Introduction

The increasing global demand for food necessitates the restructuring of agricultural production systems. Traditional methods have become inadequate, particularly due to problems such as soil degradation, water scarcity, and low productivity. In this context, artificial intelligence (AI) offers a new paradigm in agriculture through data-driven decision-making mechanisms. The continuous growth of the world population is increasing pressure on food supply. Under these circumstances, traditional agricultural production methods are insufficient to meet the growing demands in terms of efficiency, precision, and sustainability (Chen et al., 2024). In addition, cultivable agricultural lands and agricultural resources are decreasing. The aging population engaged in agriculture makes the use of modern technologies essential for increasing production potential (El Jarroudi et al., 2024). Within this framework, the rapid development of smart agricultural devices and equipment has emerged as one of the main driving forces of agricultural modernization and productivity enhancement.

The prediction of the Food and Agriculture Organization (FAO) that food production will increase by approximately 70% by 2050 (McKenzie and Williams, 2015) necessitates the adoption of innovative, sustainable, and scalable solutions in the agricultural sector. At this point, the digital transformation process referred to as Agriculture 4.0 offers significant opportunities both to increase productivity and to reduce environmental impacts (Sadiku et al., 2020). This transformation is shaped by the integration of various technologies, including artificial intelligence (AI), drones, robotics technologies, sensor systems, 5G, blockchain, and cloud computing (Melki et al., 2025). Thus, it enables data-driven decision-making, smart automation, and ecologically optimized production processes.

AI-supported agricultural applications increase production efficiency. In addition, they contribute to the effective and sustainable use of natural resources. Particularly through precision agriculture approaches, the use of water, fertilizers, and pesticides can be optimized, thereby reducing environmental pressures and preserving ecosystems (Getahun et al., 2024). To facilitate the widespread adoption of these technologies, it is important to strengthen data infrastructure, improve the digital skills of producers, and reduce costs (Wolfert et al., 2017). In such cases, the ethical, data security, and accessibility dimensions of AI applications are highly important. Consequently, AI-based solutions developed within the scope of Agriculture 4.0 play a role in ensuring food security and establishing climate-resilient systems. Therefore, increasing interdisciplinary collaboration is important for implementing this transformation effectively and inclusively.

Developments in AI applications in agriculture have emerged as a force shaping agricultural practices on a global scale. Thanks to AI technologies, the agricultural sector has achieved significant gains in terms of efficiency, productivity, and sustainability. They have also contributed to addressing challenges such as climate change, food security, and resource scarcity. Innovations such as AI-supported precision agriculture, smart irrigation systems, and autonomous equipment increase farmers' productivity while reducing input use. In this respect, AI has become one of the fundamental components of modern agricultural strategies (Nautiyal et al., 2025; Lenniy, 2023).

Developments related to the use of AI in agriculture have gained momentum, particularly since the 2000s. AI technologies analyze datasets obtained from various sources, such as sensors, unmanned aerial vehicles, and satellite systems. In this way, they support earlier target achievement and data-driven stages in agricultural activities (Sodikova, 2026).

The use of AI in agriculture also brings several important challenges. Factors such as high initial costs, concerns regarding data privacy protection, and the need for farmers to acquire adequate knowledge slow the adoption of these technologies. Furthermore, ensuring compatibility between existing agricultural practices and new technologies is a critical issue for sector stakeholders (Das et al., 2026).

Keeping pace with developments in AI applications is necessary for achieving a transformation toward smart and sustainable production systems in agricultural activities. This transformation aims not only to increase agricultural productivity but also to establish resilient agricultural systems capable of meeting the needs of the growing world population (AlZubi & Galyna, 2023; Balyan et al., 2024).

Artificial Intelligence and Agricultural Systems

Artificial intelligence is an interdisciplinary field encompassing machine learning, deep learning, and big data analytics. Traditional agricultural methods have become inadequate in the face of climate change and increasing population pressure. At this point, Artificial Intelligence (AI) has become a driving force that optimizes efficiency in agricultural production through machine learning (ML), deep learning (DL), and big data analytics (Liakos et al., 2018).

Traditional agricultural practices are being significantly transformed through the use of AI-supported autonomous agricultural equipment. Agricultural machinery equipped with artificial intelligence algorithms can operate autonomously in fields with minimal human intervention. This automation increases efficiency, reduces labor costs, and enables precision agriculture practices (Kaushal, 2024). Autonomous systems are also among the fundamental components of precision agriculture and can operate in

real time based on the data collected from their surroundings (Figure 1). In this way, agricultural activities can be carried out more accurately, timely, and effectively (Pai et al., 2025).



Figure 1. Harvest of the Future: An Artificial Intelligence-Supported Agricultural Hub

Analyzes Satellite Data

Artificial intelligence plays a critical role in the processing of multispectral and hyperspectral satellite imagery. Large-scale land analyses that traditionally require extensive time can be performed at near real-time speed through AI algorithms. With these models:

Crop Health Monitoring: AI models analyze parameters such as the Normalized Difference Vegetation Index (NDVI) using data obtained from satellites. In this way, areas under stress can be identified before visible symptoms appear. Artificial intelligence technologies play an important role in pest and disease management through early detection and targeted interventions. For example, satellite imagery can be analyzed using AI to detect pest infestations in fields at an early stage. This information is communicated to farmers through mobile notifications, enabling timely intervention without delay (Nautiyal et al., 2025; Sharma et al., 2025). In addition, Decision Support Systems (DSS) that guide farmers

regarding the most appropriate timing and methods for pest control significantly reduce pesticide use and the associated environmental impacts (Nautiyal et al., 2025).

In land classification, Convolutional Neural Networks (CNNs) provide a significant advantage. Through these methods, satellite and aerial images can be analyzed in detail, allowing different crop patterns and changes in land use to be identified with high accuracy. For example, it becomes possible to determine more clearly which crops are concentrated in a particular region or how agricultural lands have changed over time.

Beyond being merely a technical output, such data provide highly valuable information for decision-makers. The maps generated from these analyses enable more informed agricultural planning, promote the efficient use of resources, and contribute to the development of appropriate regional policies. Particularly in studies covering large areas, this approach not only saves time but also significantly reduces the difficulties associated with field data collection (Wolfert et al., 2017)

Processes Data Obtained from Sensors

Data collection in agricultural systems is one of the most important parameters that support the decision-making mechanisms of AI. Sensors that measure soil moisture, pH, temperature, and nutrient levels provide a substantial flow of data.

Smart Irrigation: AI combines moisture data obtained from sensors with weather forecasts to establish “smart irrigation” schedules. This approach can contribute to water conservation and the sustainable management of water resources.

Precision Fertilization: Variable Rate Application (VRA) technology utilizes sensor data to apply fertilizers only to the areas of a field where they are required. In this way, input costs are reduced

while groundwater contamination can be prevented (Kamilaris et al., 2018).

Data obtained from sensors in agricultural systems constitute a fundamental source of information that enables artificial intelligence to make accurate and reliable decisions. Through sensors that measure soil moisture, pH, temperature, and nutrient elements, a continuous and real-time flow of field-related data is generated. This allows producers to evaluate their fields not only through observation but also on the basis of data-driven insights.

AI-supported approaches are particularly prominent in irrigation management. The integration of moisture data from sensors with weather forecasts enables more accurate determination of irrigation timing and quantity. As a result, unnecessary water use can be avoided while ensuring that crops receive sufficient irrigation precisely when needed. In arid and semi-arid regions, such applications provide significant advantages for the conservation of water resources.

Similarly, substantial transformations have occurred in fertilization practices. Through Variable Rate Application (VRA) technology, fertilizers can be applied at appropriate rates only to the areas requiring treatment, rather than uniformly across the entire field based on sensor-derived data. This approach not only reduces input costs but also helps prevent environmental problems associated with excessive fertilizer use, particularly groundwater contamination (Kamilaris et al., 2018).

Develops Predictive Models

One of the most strategic contributions of AI in agriculture is its ability to learn from previously collected data and generate predictions for future conditions. In this way, producers not only evaluate current situations but can also anticipate potential risks and act in a more planned manner.

For example, artificial intelligence is a highly powerful tool for yield prediction. Big data analytics can evaluate soil characteristics, seed varieties used, and historical climate data simultaneously to predict crop yield at harvest with high accuracy. Such predictions provide farmers with significant advantages in both production planning and the development of marketing strategies.

Similarly, artificial intelligence functions as an early warning system in the management of diseases and pests. Moisture and temperature data obtained from sensors can be analyzed using deep learning algorithms to identify, in advance, periods with a high risk of fungal diseases or insect infestations. As a result, producers have the opportunity to take preventive measures before problems arise, while unnecessary pesticide applications can also be avoided (Klerkx et al., 2019).

Precision Agriculture and Soil Management

Analysis of Soil Properties

Through AI-supported sensor technologies, the physical, chemical, and biological properties of soil can now be monitored in real time. This enables producers to evaluate their fields not only through periodic analyses but also through a continuous flow of data.

Within this framework, key parameters such as soil moisture, organic matter content, pH, electrical conductivity (EC), and microbial activity can be monitored regularly. The data obtained provide a clearer and more dynamic picture of the current condition of the soil, while also enabling the tracking of changes occurring over time.

Such a monitoring system contributes significantly to more accurate irrigation and fertilization decisions. For example, when soil moisture status is known in real time, unnecessary irrigation can be avoided, while applications based on nutrient levels can both

increase productivity and reduce environmental impacts. Furthermore, monitoring microbial activity provides an important advantage for evaluating the biological health of the soil.

Microbial Biomass and AI

Soil microbial biomass (MBC and MBN) is one of the most important indicators of soil health. Soil microbial biomass (MBC and MBN) serves as a key indicator of soil vitality and overall soil condition. In this context, artificial intelligence models can monitor changes in microbial biomass and generate predictions regarding future trends.

Furthermore, the effects of biochar applications on soil can be analyzed more clearly, and changes in carbon and nitrogen cycling can be better understood. As a result, soil processes can be evaluated in a more holistic and data-driven manner.

Autonomous Systems and Robotic Agriculture

Autonomous Tractors and Robotic Systems: Autonomous tractors are among the most important components of modern agricultural automation. These vehicles are equipped with GNSS-based navigation systems, enabling them to perform conventional tractor operations such as plowing, seeding, and soil cultivation without human intervention (Chen, 2025) (Figure 2).

Existing manual tractors can also often be retrofitted with autonomous capabilities. Sensors integrated into these systems detect obstacles in the field, helping to prevent collisions and ensuring a safe working environment (Bhat and Wang, 2025).



Figure 2. Artificial Intelligence in Precision Agriculture: Soil Management Map

Robotic Harvesting Systems

Automation in harvesting operations has made significant progress in recent years, particularly for sensitive crops such as fruits, vegetables, and nuts. Robotic harvesting systems are being developed to move through fields, identify mature products, and perform harvesting and sorting operations. However, this task is not as simple as it appears. Accurately distinguishing whether a fruit has reached maturity under varying lighting conditions and handling it delicately without causing damage during harvesting are among the major technical challenges (Mann & Könnö, 2025).

Applications such as Agrobot's strawberry-picking robot and FFRobotics' apple harvesting system combine artificial intelligence and robotic technologies to reduce crop losses while alleviating the workload of farmers, particularly during labor-intensive harvesting periods.

Pest and Disease Management Robots

Autonomous systems also play an important role in pest and disease management, which directly affects agricultural productivity and crop quality. Through artificial intelligence and robotic technologies, pesticide application processes can be carried out more rapidly and efficiently. Interventions are performed only in areas where problems are detected (Gackstetter et al., 2023).

Since these robots can detect and continuously monitor pests, they are able to intervene only at the required locations, thereby reducing unnecessary chemical use and contributing to healthier plant development. Today, increasing environmental concerns and the decline of the agricultural workforce are making traditional methods progressively more difficult to implement. For this reason, the integration of robotic systems into agriculture is accelerating and plays a significant role in meeting the need for more sustainable production methods (Obeng-Ofori et al., 2025).

Climate Data and Yield Prediction

AI-based models provide:

- Drought analysis
- Rainfall prediction
- Crop yield simulation

The interpretation of climate data and the prediction of crop yields have become significantly more understandable and practical in agriculture through artificial intelligence. These systems integrate data such as temperature, precipitation, and soil moisture to identify drought risks in advance, predict the timing and amount of rainfall, and estimate crop performance under different environmental conditions. By learning from historical datasets, these models provide guidance to farmers on critical decisions, such as determining optimal planting times and appropriate irrigation schedules. As a result, more informed decisions can be made, and

agricultural production systems can become more resilient to the uncertainties associated with climate change.

Smart Irrigation and Water Management

The soil–water–plant relationship is optimized through AI:

- Real-time irrigation
- Water-use efficiency
- Plant stress analysis

Smart irrigation systems have been developed using artificial intelligence technologies to ensure more efficient water use in agriculture. These systems analyze data obtained from soil moisture sensors and weather forecasts and provide plants with the precise amount of water they need at the appropriate time. In this way, unnecessary water consumption is prevented while promoting healthier plant growth. As a result, water savings are achieved, productivity is enhanced, and a more effective solution is provided for the challenges posed by climate change and water scarcity (Nautiyal et al., 2025).

AI-integrated smart irrigation systems demonstrate significant potential for improving agricultural productivity. Through the use of sensors and Internet of Things (IoT) technologies, farmers can continuously monitor variables such as field temperature and crop water requirements. The automation of agricultural processes enables higher yields to be achieved with reduced water and fertilizer inputs, thereby supporting sustainability in agricultural production systems (Nautiyal et al., 2025; Reddy, 2022).

Big Data and Decision Support Systems

AI systems:

- Integrate data from multiple sources

- Provide recommendations to farmers
- Minimize risks

Big data and decision support systems constitute the foundation of artificial intelligence applications that are becoming increasingly important in agriculture. These systems generate meaningful insights by integrating information from multiple sources, including satellite imagery, weather data, soil sensors, and historical production records. They not only provide data to farmers but also offer practical recommendations regarding planting schedules, irrigation amounts, fertilizer application, and disease risks (Klerkx et al., 2019). As a result, uncertainties are reduced, production becomes more systematic, and potential risks are minimized. Particularly in an era of increasing climate variability, such decision support systems enhance productivity while contributing to the more efficient and informed use of resources (Figure 3).

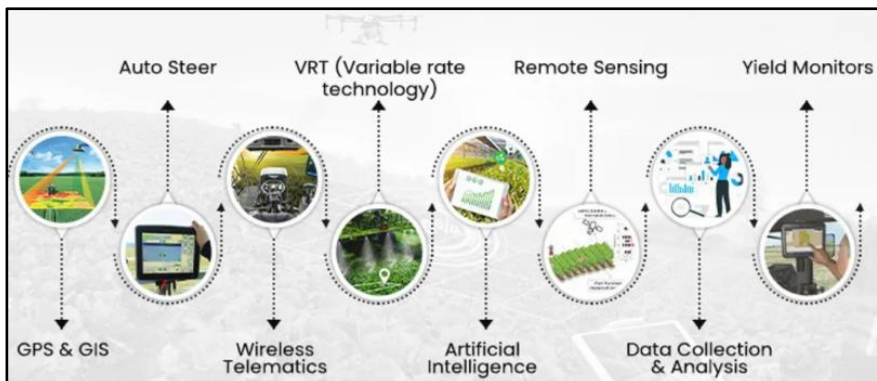


Figure 3. Precision Agriculture Diagram: Artificial Intelligence, Data, and Decision Support Systems

Advantages and Limitations

Advantages

- Increased productivity
- Resource optimization
- Environmental sustainability

Disadvantages

- High cost
- Data deficiency
- Technical infrastructure problems

Artificial intelligence-based agricultural technologies offer numerous important advantages, making production processes more efficient and sustainable. Through these systems, productivity can be increased while resources such as water, fertilizers, and energy can be utilized more effectively. At the same time, agricultural production becomes more sustainable through the reduction of environmental impacts. However, these technologies also have certain limitations. High installation and operational costs constitute a significant barrier, particularly for small-scale producers, while the lack of sufficient and high-quality data can negatively affect system accuracy. In addition, inadequate technical infrastructure in rural areas remains another major factor limiting the widespread adoption of these technologies.

The strong dependence on large datasets in precision agriculture also brings several important challenges related to data quality and representativeness. Biased or insufficiently representative datasets may lead to the development of biased artificial intelligence models, which can negatively affect all stakeholders, particularly disadvantaged or marginalized groups (Gardezi et al., 2024).

Furthermore, conventional data-processing methods may be insufficient to meet the increasing data requirements of smart

agriculture. Therefore, the use of more advanced techniques, such as machine learning, is becoming increasingly necessary to extract meaningful and valuable insights from data (Hasim et al., 2025).

Conclusion

Artificial intelligence is a key driver of sustainable agricultural production. In particular, its integration with soil science will enable the development of more efficient and environmentally friendly systems in the future.

This study demonstrates that artificial intelligence is not merely a technology in agriculture but also a powerful tool that transforms production practices. Through AI-supported applications, agricultural production processes are becoming more efficient, precise, and sustainable. In particular, the combined use of sensors, satellite data, and autonomous systems provides farmers with opportunities to make more accurate and timely decisions.

However, this transformation process also brings several challenges. High costs, data deficiencies, inadequate technical infrastructure, and the adaptation of users to new technologies are among the primary factors limiting the widespread adoption of artificial intelligence. In addition, issues related to data security and equitable access should not be overlooked.

In the future, the effective utilization of artificial intelligence in agriculture will depend on interdisciplinary collaboration, the development of appropriate policies, and improved accessibility of these technologies for farmers. In this way, food security can be strengthened while more resilient agricultural systems can be developed to address the challenges posed by climate change.

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CHAPTER 2

WHAT IS GREEN CARBON AND WHAT IS IT NOT?

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Introduction

Over the past two decades, the role of terrestrial ecosystems in regulating atmospheric carbon dioxide concentrations has received growing scientific and policy attention. Although fossil fuel emissions remain the primary driver of contemporary climate change, it is now widely recognized that land-based ecosystems exert a critical influence on the global carbon balance. Through their capacity to absorb, store, and release carbon, terrestrial systems can either mitigate or intensify ongoing climatic trends, depending on environmental conditions and management practices (Rayner et al., 2005).

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Within this context, the concept of green carbon has emerged as a unifying framework for describing biologically mediated carbon storage in terrestrial environments (Wang et al., 2025).

This chapter approaches green carbon as an integrative concept that links vegetation, soils, and land use within the broader functioning of the global carbon cycle. Rather than viewing carbon storage as a static property of forests, grasslands, or soils, the chapter adopts a dynamic perspective that emphasizes carbon fluxes, stabilization mechanisms, and system vulnerabilities (Zhou et al., 2021). Attention is given to the processes through which carbon moves among ecosystem components and to the feedbacks that connect terrestrial carbon dynamics with climate variability and human disturbance (Ciais et al., 2014).

The discussion encompasses a wide range of terrestrial ecosystems, including forests, grasslands, wetlands, and agricultural soils, reflecting their contrasting capacities for carbon storage and their differing sensitivities to environmental change (Lal et al., 2018). While marine carbon processes and fossil carbon reservoirs are not treated in detail, they are referenced where necessary to provide conceptual contrast and to situate green carbon within the larger carbon cycle.

The chapter is intended for readers with backgrounds in soil science, ecology, environmental science, and land management, but it is also designed to be accessible to those engaged in climate policy, carbon accounting, and applied sustainability planning. By combining process-based understanding with management and

policy relevance, the chapter aims to clarify the role of green carbon in climate regulation and to highlight both its potential and its limitations as a component of climate mitigation strategies.

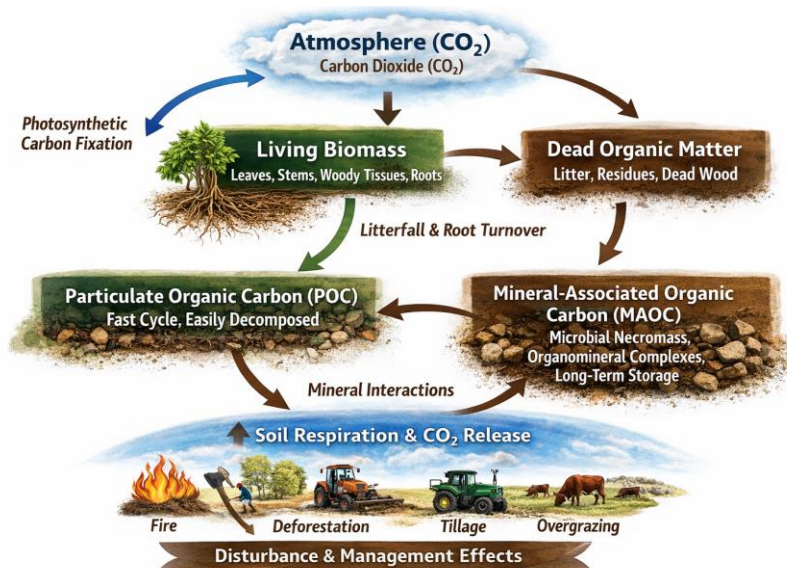
Concept and Definition of Green Carbon

Green carbon refers to carbon captured from the atmosphere through photosynthesis and subsequently stored within terrestrial ecosystems (Earth et al., 2018). This carbon is distributed across multiple interconnected pools, including living plant biomass, dead organic matter such as litter and woody debris, and soil organic carbon. The concept of green carbon is commonly used to distinguish biologically mediated terrestrial carbon from fossil-derived carbon and from carbon stored in marine and coastal systems (Mackey et al., 2008).

Unlike fossil carbon, green carbon is inherently dynamic. It is continuously exchanged between the biosphere and the atmosphere through biological and biogeochemical processes, and its storage is therefore reversible. Carbon accumulated over years or decades can be released within relatively short timeframes through land-use change, fire, or soil disturbance. This reversibility makes green carbon both a valuable component of climate regulation and a fragile one (Kooten, 2009).

As illustrated in Figure 1, green carbon does not reside in a single reservoir but circulates continuously among vegetation, dead organic matter, and soil carbon pools (Schlamadinger et al., 2007). Atmospheric carbon dioxide enters terrestrial ecosystems through photosynthetic carbon fixation, whereby plants incorporate inorganic carbon into leaves, stems, woody tissues, and roots. Carbon stored in living biomass is subsequently transferred to the soil system through aboveground litterfall and belowground root turnover, forming the pool of dead organic matter composed of plant residues, litter, and dead wood.

Figure 1. Conceptual framework of green carbon dynamics.



During decomposition, carbon derived from dead organic matter is further partitioned into two functionally distinct soil organic carbon fractions. Particulate organic carbon consists of relatively fresh or partially decomposed organic materials that are only weakly associated with soil minerals. This fraction exhibits rapid turnover and is highly sensitive to microbial activity and physical disturbance (Zhu et al., 2010). In contrast, mineral-associated organic carbon is formed primarily through microbial processing and subsequent interactions between organic compounds and fine mineral surfaces. This fraction is physically and chemically protected, turns over more slowly, and represents the dominant pool responsible for long-term carbon storage in soils (Bastos et al., 2022; Yanardağ et al., 2021). Soil microbial communities play a pivotal role in these processes, actively participating in the decomposition and transformation of organic matter and contributing to the stabilization of organic carbon (Liang et al., 2025). The rate of carbon turnover depends on environmental conditions, soil

management, and soil component interactions (Yanardağ et al., 2014). Stable organic compounds can be physically preserved by their adsorption on mineral surfaces or bound in aggregates (Yanardağ et al., 2021).

Carbon is continuously returned to the atmosphere through soil respiration, reflecting the combined metabolic activity of plant roots and soil microorganisms. Although both soil carbon fractions contribute to CO₂ release, emissions are typically dominated by the more labile particulate pool, particularly under conditions that promote decomposition. The framework also highlights the role of disturbance and management factors, including fire, deforestation, tillage, and overgrazing. These pressures can disrupt green carbon dynamics by accelerating carbon losses from vegetation and soil, preferentially depleting labile carbon pools and limiting the formation and persistence of mineral-associated carbon. Consequently, green carbon storage is governed not only by ecological and biogeochemical processes, but also by land-use decisions and management practices (Vera et al., 2022). Overall, green carbon represents a dynamic and interconnected system in which short-term biological fluxes coexist with longer-term stabilization mechanisms. The balance between these processes ultimately determines the capacity of terrestrial ecosystems to function as effective and resilient carbon sinks.

Green Carbon in the Global Carbon Cycle

Within the global carbon cycle, green carbon represents a major interface between atmospheric carbon dioxide and longer-term terrestrial storage (Wang et al., 2025). Each year, terrestrial ecosystems absorb a substantial fraction of anthropogenic CO₂ emissions, temporarily slowing the accumulation of greenhouse gases in the atmosphere. This land sink, however, is neither constant nor guaranteed (Friedlingstein et al., 2025).

Interannual variability in atmospheric CO₂ growth rates is strongly influenced by changes in terrestrial carbon uptake, driven by climate anomalies such as droughts, heatwaves, and extreme precipitation events. These variations highlight the sensitivity of green carbon to climatic conditions and underscore its role as an active, responsive component of the Earth system (Luo & Keenan, 2022).

Unlike geological carbon reservoirs, terrestrial carbon stocks are closely coupled to biological activity. Photosynthesis, respiration, decomposition, and disturbance collectively determine whether ecosystems function as net carbon sinks or sources (Lal et al., 2018). As climate change alters these processes, the stability of green carbon becomes increasingly uncertain, with important implications for future climate trajectories (Roebroek et al., 2024). Understanding the impact of climate change on soil organic carbon dynamics is critical for assessing future climate change mitigation potential (Sokol et al., 2022).

Major Ecosystems Storing Green Carbon

Terrestrial ecosystems differ markedly in both the magnitude and the distribution of green carbon among biomass, dead organic matter, and soil pools. These differences reflect contrasting vegetation traits, decomposition environments, disturbance regimes, and land-use histories, and they strongly influence ecosystem-level carbon stability.

Forests are often regarded as archetypal green carbon ecosystems due to their large standing biomass and the longevity of woody tissues (Mo et al., 2023). Carbon is stored not only in living trees but also in dead wood, litter layers, and forest soils. While aboveground biomass represents a prominent and visible carbon pool, soils frequently contain a substantial proportion of total forest carbon, particularly in mature or minimally disturbed systems. The

relative contribution of these pools varies with forest age, climate, and management intensity (O'Sullivan et al., 2022).

Grasslands and rangelands, although characterized by comparatively low aboveground biomass, play a critical role in green carbon storage through their extensive belowground systems (Ren et al., 2024). Dense root networks and continuous root turnover promote the accumulation of soil organic carbon, often making soils the dominant carbon reservoir in these ecosystems. Under appropriate grazing and management regimes, grasslands can maintain stable or even increasing soil carbon stocks despite limited aboveground carbon accumulation.

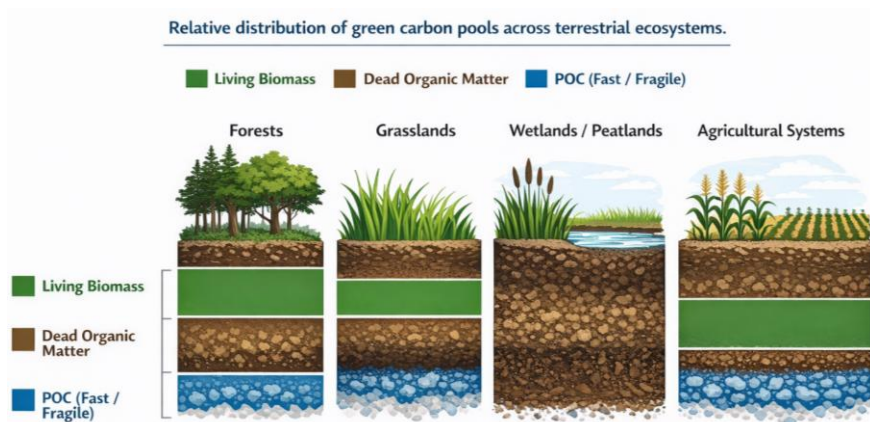
Wetlands and peatlands represent some of the most carbon-rich terrestrial ecosystems. Persistent water saturation and oxygen limitation slow organic matter decomposition, allowing carbon to accumulate over centuries or millennia in the form of peat and organic-rich soils (Brander & Broekhoff, 2023; Dooley et al., 2024). Despite their relatively small global extent, these ecosystems store disproportionate amounts of green carbon and are particularly vulnerable to drainage, land conversion, and hydrological alteration, which can rapidly transform long-term carbon sinks into major sources of atmospheric CO₂. Wetlands play a key role in climate regulation and carbon sequestration, making their ability to sequester carbon important for national greenhouse gas assessments (Moomaw et al., 2018).

Agricultural systems occupy a distinct position within the green carbon framework. Cultivation, residue removal, and frequent soil disturbance have often resulted in substantial losses of original soil carbon stocks. Consequently, agricultural landscapes are typically characterized by smaller and more dynamic carbon pools, dominated by fast-cycling soil organic matter (Yanardağ et al., 2021). At the same time, agricultural soils offer considerable

potential for carbon restoration through improved management practices such as reduced tillage, residue retention, cover cropping, and organic amendments, making them central to strategies aimed at enhancing green carbon storage.

As illustrated in Figure 2, these ecosystems differ not only in total carbon storage but also in the relative dominance of biomass versus soil-based carbon pools. Such contrasts have important implications for carbon turnover rates, vulnerability to disturbance, and the design of land-use strategies targeting climate mitigation through green carbon management.

Figure 2. Relative distribution of green carbon among major terrestrial ecosystems.



Schematic representation of the dominant green carbon pools in forests, grasslands, wetlands/peatlands, and agricultural systems. The figure illustrates ecosystem-specific patterns of carbon allocation, with forests characterized by large stocks of long lived living biomass, grasslands by predominantly soil-based carbon derived from extensive root systems, wetlands and peatlands by the accumulation of dead organic matter under anaerobic conditions, and agricultural systems by relatively small and highly dynamic carbon pools dominated by fast cycling particulate organic carbon (POC).

The relative thickness of each pool reflects differences in turnover rates, stability, and sensitivity to disturbance rather than absolute carbon quantities.

Threats, Vulnerabilities, and Climate Feedback

Green carbon stocks are inherently sensitive to both direct human disturbance and indirect climatic pressures. Land-use activities such as deforestation, land conversion, intensive tillage, overgrazing, and wetland drainage can rapidly transform long-term carbon sinks into net sources of atmospheric CO₂. In most terrestrial ecosystems, the rate of carbon loss following disturbance far exceeds the rate at which carbon can be re-accumulated, resulting in prolonged carbon deficits (Dobor et al., 2018). Human impacts are often the main driver of tropical forest carbon loss (Pyles et al., 2022).

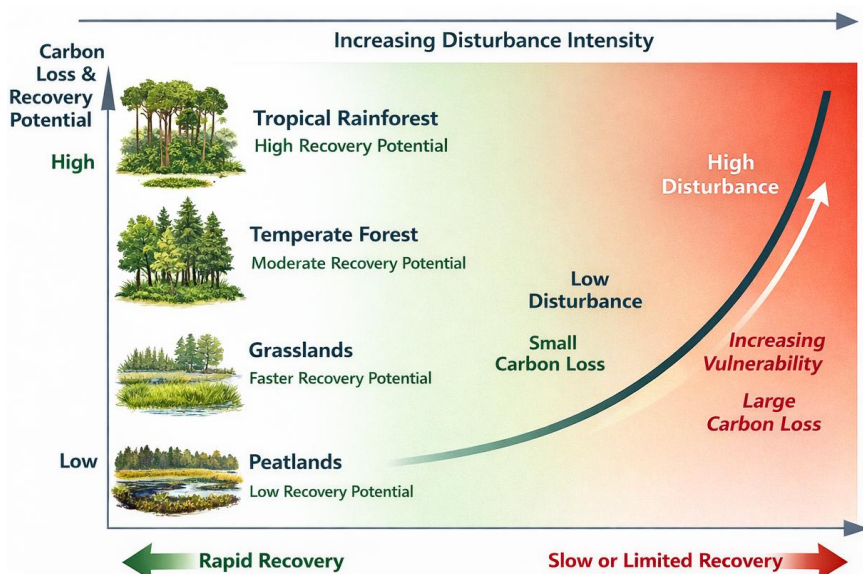
The vulnerability of green carbon is not uniform across ecosystems but varies strongly with ecosystem type, carbon pool composition, and disturbance history (Tadese et al., 2023). As conceptually illustrated in Figure 3, systems dominated by stable soil carbon pools tend to exhibit greater resistance to low levels of disturbance, whereas ecosystems relying heavily on long-lived organic matter or hydrologically protected carbon can experience abrupt and often irreversible losses once critical thresholds are crossed. Peatlands, in particular, display extremely low reversibility following disturbance, reflecting the long timescales required for peat formation. Disturbances like biomass harvest and intensive grazing diminish the potential of forests to store carbon (Dar & Parthasarathy, 2022).

Climate change introduces additional layers of vulnerability that interact with land-use pressures. Rising temperatures generally enhance microbial activity, accelerating organic matter decomposition and soil respiration, while altered precipitation

regimes influence plant productivity, soil moisture, and oxygen availability (Bamière et al., 2022; Yanardağ et al., 2021). These changes can either constrain carbon inputs or stimulate carbon losses, depending on ecosystem context (Varney et al., 2023). Furthermore, the increasing frequency and intensity of extreme events such as droughts, heatwaves, and wildfires amplify the likelihood of sudden and large carbon releases from both biomass and soil pools (Tadese et al, 2023). Climate change also pushes tree populations to the edge of their natural climate envelopes, increasing risks of tree mortality and potentially turning carbon sinks into sources (Roebroek et al., 2023).

Together, these processes generate powerful feedbacks within the climate system. Carbon released from terrestrial ecosystems contributes to further atmospheric warming, which in turn promotes additional carbon losses by intensifying decomposition, disturbance frequency, and ecosystem stress. This positive feedback loop underscores the central challenge of green carbon management: preventing carbon loss is often far more effective than attempting to restore carbon stocks after disturbance has occurred.

Figure 3. Vulnerability and reversibility of green carbon stocks under disturbance.



This figure conceptualizes the relationship between disturbance intensity and green carbon vulnerability across terrestrial ecosystems. The horizontal axis represents a gradient of disturbance intensity, ranging from low to high, encompassing increasing disturbance frequency, magnitude, and irreversibility. The vertical axis reflects the combined outcome of carbon loss magnitude and recovery potential, emphasizing that vulnerability depends not only on how much carbon is lost, but also on how readily it can be restored.

At low disturbance levels, ecosystems experience relatively limited carbon losses and retain high reversibility. In this domain, biological processes such as vegetation regrowth, litter inputs, root turnover, and microbial stabilization enable relatively rapid recovery of carbon stocks. As disturbance intensity increases, however, vulnerability rises non-linearly. The curved trajectory highlights

threshold behaviour, whereby incremental increases in disturbance can trigger disproportionately large carbon losses and sharply reduce recovery potential, particularly for soil carbon pools.

Ecosystem types occupy distinct positions along this disturbance vulnerability gradient. Tropical forests store large amounts of carbon in living biomass and can exhibit relatively high recovery potential under moderate disturbance due to rapid productivity, yet severe disturbance can lead to abrupt and extensive carbon losses. Temperate forests show intermediate vulnerability, with slower recovery linked to lower productivity and longer soil carbon turnover times. Grasslands generally lose less aboveground carbon and may recover more rapidly because a substantial fraction of their carbon is stored belowground, although repeated disturbance progressively degrades soil carbon stocks. Peatlands represent the most vulnerable systems: once disturbed, particularly through drainage, they experience massive carbon losses and exhibit extremely limited reversibility on human timescales.

Overall, the figure emphasizes a central principle of green carbon dynamics: vulnerability increases and reversibility declines with rising disturbance intensity, and this trade-off is strongly ecosystem specific. Maintaining low disturbance states and avoiding critical thresholds is therefore considerably more effective for climate mitigation than relying on post-disturbance restoration strategies.

Measurement, Monitoring, and Modelling Approaches

Quantifying green carbon and understanding its dynamics require an integrated framework that combines direct measurements, observational monitoring, and process-based modelling. No single method is sufficient to capture the complexity of green carbon systems, as carbon stocks and fluxes vary across spatial scales, ecosystem types, and time horizons. Consequently, robust

assessment depends on the complementary use of field observations, laboratory analyses, remote sensing technologies, and modelling approaches (West et al., 2013).

Field-based measurements

Field measurements remain the foundation of green carbon assessment. Biomass inventories provide direct estimates of carbon stored in living vegetation through measurements of tree diameter, height, and wood density in forests, or through destructive and non-destructive sampling in grasslands and agricultural systems. Belowground biomass, although more difficult to quantify, is critical in ecosystems where root-derived carbon dominates soil inputs.

Soil sampling is essential for assessing soil organic carbon stocks and their vertical distribution. Measurements typically involve sampling to standardized depths, followed by laboratory determination of carbon concentration and bulk density. Increasingly, soil carbon assessments distinguish between functionally distinct fractions, such as particulate organic carbon and mineral-associated organic carbon, to better capture differences in turnover rates and stabilization mechanisms (Yanardag et al., 2021). However, spatial heterogeneity, sampling depth limitations, and temporal variability remain major sources of uncertainty in soil carbon estimates.

To complement stock measurements, flux-based approaches provide insights into the rates at which carbon moves through ecosystems. Techniques such as chamber-based measurements and eddy covariance systems quantify carbon dioxide exchange between land surfaces and the atmosphere, enabling the assessment of net ecosystem carbon balance (Luo & Keenan, 2022). While flux measurements capture short term dynamics and responses to environmental variability, they require careful interpretation when extrapolated to longer time scales.

Laboratory analyses and process indicators

Laboratory analyses play a key role in characterizing green carbon quality and stability. Fractionation techniques, isotopic analyses, and microbial indicators are increasingly used to elucidate carbon sources, transformation pathways, and stabilization processes (Yanardağ et al., 2017). Enzyme activities, microbial biomass, and respiration measurements provide additional insight into the biological controls on carbon turnover (Baydilli et al., 2025; Sakin et al., 2024). Although these approaches enhance mechanistic understanding, they are often site-specific and challenging to scale up.

Remote sensing and spatial monitoring

Advances in remote sensing have greatly expanded the capacity to monitor green carbon at regional to global scales (Salem, 2025). Satellite-based observations of vegetation structure, productivity, and phenology enable indirect estimation of aboveground biomass and carbon uptake, particularly in forested systems. Optical sensors, radar, and lidar technologies have improved the accuracy of biomass mapping and disturbance detection. Remote sensing allows for cost-effective, large-scale studies of forest carbon budgeting and tracking changes over time due to disturbances or afforestation efforts (Villa & Bernal, 2017; Yanardağ et al., 2021).

In contrast, soil carbon remains far more difficult to assess remotely, as it is influenced by subsurface properties that are not directly observable from space. As a result, spatially explicit soil carbon estimates often rely on a combination of remote sensing proxies, digital soil mapping, and ground-based calibration data. Uncertainties are especially pronounced in heterogeneous landscapes and managed ecosystems, where land use and management practices vary over short distances.

Modelling approaches and integration

Models play a central role in synthesizing observations, exploring scenarios, and projecting future green carbon dynamics (Reichle et al., 1999). Empirical, semi empirical, and process-based models are used to simulate carbon stocks and fluxes under different climatic and management conditions. These models help bridge gaps between short term measurements and long-term carbon trajectories, providing a basis for scenario analysis and policy-relevant assessments (Scholze et al., 2017).

However, model performance is strongly constrained by data availability and the representation of key processes, particularly those governing soil carbon stabilization, microbial dynamics, and disturbance impacts (Munro et al., 2025). Uncertainty in model outputs often reflects limited understanding of belowground processes and insufficient long-term observations. As a result, model-based estimates of green carbon should be interpreted as conditional projections rather than precise predictions.

Toward integrated assessment frameworks

Effective assessment of green carbon ultimately depends on long-term monitoring and cross-scale integration. Linking field measurements with remote sensing products and models allows for more robust evaluation of carbon dynamics and uncertainty. Such integrated frameworks are increasingly important for carbon accounting, climate mitigation planning, and the evaluation of land-use strategies aimed at enhancing green carbon storage.

Land-Use and Management Strategies to Enhance Green Carbon

Land-use and management decisions exert a decisive influence on the magnitude, stability, and longevity of green carbon stocks across terrestrial ecosystems (Levy et al., 2024). Management

interventions can either enhance net carbon inputs and promote stabilization in long lived pools or accelerate carbon losses by increasing disturbance and decomposition. Consequently, effective green carbon enhancement requires land-use-specific strategies that account for ecosystem structure, dominant carbon pools, and vulnerability to disturbance (Papa et al., 2023).

In forested systems, conserving intact forests remains the most effective strategy for maintaining large, long-lived carbon stocks. Extending harvest rotations, reducing logging intensity, and restoring degraded forest areas can further enhance carbon storage by increasing standing biomass and sustaining litter and dead wood inputs to soils (Coşofreţ et al., 2024). Although afforestation and reforestation can contribute to additional carbon sequestration, their effectiveness depends strongly on site conditions, prior land use, and species selection, and carbon gains are not universally guaranteed (Xu et al., 2022).

In agricultural landscapes, management pathways focus primarily on rebuilding soil organic carbon stocks that have often been depleted by cultivation and residue removal. Practices such as reduced or no-till management, residue retention, cover cropping, and the use of organic amendments increase organic inputs and improve soil aggregation, thereby favouring the transfer of carbon into more stable soil pools (Bamière et al., 2022). Agroforestry systems are particularly effective, as they combine aboveground biomass accumulation with sustained belowground carbon inputs and reduced erosion. Additionally, planting perennial bioenergy crops on low carbon or marginal lands can enhance soil carbon sequestration and contribute to climate change mitigation. However, large scale expansion of bioenergy crops may lead to competition for land and potential indirect land-use change emissions (Vera et al., 2022). The use of biochar as a soil enhancer can also help balance

CO₂ emissions and increase long term carbon sequestration (Clough et al., 2013; Sakin & Yanardağ, 2019; Sakin & Yanardağ, 2021).

Grassland management strategies emphasize maintaining continuous plant cover and enhancing belowground carbon allocation. Avoiding overgrazing, promoting plant species diversity, restoring degraded rangelands, and implementing adaptive grazing regimes support root production, soil structure, and aggregate stability. Together, these processes contribute to the maintenance or gradual accumulation of soil carbon, even in systems with relatively low aboveground biomass.

In wetlands and peatlands, management priorities differ fundamentally from those of other ecosystems. These systems store exceptionally high amounts of green carbon in dead organic matter and organic-rich soils formed under anaerobic conditions. Preventing drainage, conserving intact peatlands, and restoring natural hydrology through rewetting are among the most effective strategies for avoiding large and often irreversible carbon losses (Brander & Broekhoff, 2023). While wetland restoration can involve trade-offs related to methane emissions, careful hydrological and vegetation management can optimize net climate benefits while delivering additional water-quality and biodiversity co-benefits.

Urban areas, though often overlooked in carbon strategies, also offer opportunities for green carbon enhancement. Expanding urban green spaces, increasing tree cover, implementing green roofs and rain gardens, and reducing soil sealing can create localized carbon sinks while simultaneously improving microclimate regulation, habitat connectivity, and human well-being (Moon et al., 2024). Although per area carbon stocks are typically smaller than in natural ecosystems, the cumulative benefits at city scales can be substantial.

As summarized in Figure 4, effective land-use management for green carbon enhancement is inherently context dependent. Strategies must balance increasing carbon inputs, reducing losses from disturbance, and protecting existing high-carbon ecosystems, particularly wetlands and peatlands, where prevention of degradation is far more effective than post disturbance restoration.

Figure 4. Land-use management pathways for enhancing green carbon.



This figure presents a conceptual framework summarizing land-use specific management pathways that enhance green carbon through three complementary mechanisms: (i) increasing net carbon inputs via biomass production, residue return, and rhizodeposition; (ii) reducing carbon losses associated with erosion, oxidation, and accelerated decomposition; and (iii) strengthening ecosystem functions that stabilize carbon in long-lived pools.

The figure is organized into five columns representing dominant land-use contexts: forests, agricultural lands, grasslands, wetlands, and urban areas. In each column, the upper panel highlights priority management strategies commonly applicable

within that land-use type, while the lower panel synthesizes the expected outcomes for green carbon storage and associated co-benefits.

In forests, pathways emphasize reforestation and afforestation, improved forest management, and reduced deforestation to increase standing biomass, sustain dead organic matter inputs, and protect soil carbon by limiting disturbance. In agricultural systems, cover cropping, reduced or no-till management, and agroforestry are shown to enhance soil carbon inputs, improve soil structure, and shift carbon toward more stable pools while reducing erosion and fuel-related emissions. Grassland pathways focus on restoration with native species, adaptive grazing management, and fire-risk management to maintain continuous belowground carbon allocation and minimize soil exposure. Wetland and peatland pathways prioritize conservation, restoration, and rewetting to prevent oxidative carbon losses, sustain anaerobic conditions that slow decomposition, and deliver water-quality co-benefits, while acknowledging the need to manage methane trade-offs. In urban areas, the expansion of green spaces, tree planting, and nature based infrastructure such as green roofs and rain gardens is shown to create urban carbon sinks and improve ecosystem functioning.

Directional arrows at the bottom of the figure indicate the dominant outcome class associated with each pathway: increased carbon sequestration, reduced greenhouse gas emissions, and improved ecosystem health and biodiversity. Overall, the framework highlights that successful green carbon enhancement depends on aligning management strategies with ecosystem-specific carbon dynamics and prioritizing the protection of existing high carbon systems.

Green Carbon in Climate Policy and Carbon Accounting

Green carbon has become increasingly prominent in climate mitigation frameworks, particularly through the growing emphasis on nature-based solutions, land-use change mitigation, and ecosystem restoration strategies (Marvin et al., 2023). Unlike purely technological mitigation options, green carbon offers the possibility of delivering multiple co-benefits, including improved soil health, enhanced biodiversity, increased ecosystem resilience, and socio-economic benefits for rural communities. As a result, terrestrial carbon sequestration has been integrated into national climate strategies, voluntary carbon markets, and international reporting frameworks (Tebeje, 2020).

Within climate policy, green carbon is primarily addressed through land-use, land-use change, and forestry accounting, where changes in biomass and soil carbon stocks are translated into national greenhouse gas inventories. These accounting systems increasingly recognize the importance of both vegetation and soil carbon pools, reflecting advances in scientific understanding of terrestrial carbon dynamics (Papa et al., 2023). However, the dynamic and reversible nature of green carbon presents challenges that are fundamentally different from those associated with fossil carbon mitigation (Kooten, 2009).

One of the central challenges in incorporating green carbon into climate policy is measurement uncertainty. Carbon stocks and fluxes vary widely across landscapes and over time, particularly in soils, where changes occur slowly and are strongly influenced by management history, climate variability, and inherent soil properties. Detecting meaningful changes in soil carbon at policy-relevant timescales remains difficult, increasing uncertainty in reported mitigation outcomes and complicating verification procedures (Leoni et al., 2025).

Closely related to uncertainty is the issue of permanence. Green carbon stored in biomass or soils can be rapidly released through disturbance events such as fire, drought, land conversion, or management shifts (Kooten, 2009). This reversibility raises concerns about the long-term effectiveness of green carbon credits and has led to the development of safeguards such as buffer pools, discounting factors, and long-term monitoring requirements in carbon market mechanisms (Wheeler et al., 2026). Nevertheless, the risk of carbon reversal cannot be fully eliminated and must be explicitly acknowledged in policy design.

Another key concern is the potential for unintended land-use changes. Incentivizing carbon sequestration in one location may displace agricultural production or resource extraction to other areas, resulting in indirect emissions that offset local carbon gains. Such leakage effects are particularly relevant in regions where land is under competing pressures for food production, conservation, and development. Effective policy frameworks therefore require integrated land-use planning approaches that balance carbon objectives with food security and livelihoods. Policies and programs for soil carbon sequestration must address structural issues and barriers to adoption and should be practice and place-specific rather than one-size fits-all (Roebroek et al., 2024).

Given these challenges, effective integration of green carbon into climate policy depends on scientifically credible monitoring, reporting, and verification systems that are transparent, conservative, and adaptable. Long-term commitments are essential, as short-term project horizons are poorly aligned with the timescales of soil carbon stabilization and ecosystem recovery. Policies that prioritize the protection of existing high-carbon ecosystems such as intact forests and peatlands are generally more robust and cost-effective than those relying solely on post-degradation restoration. The economic benefits of tackling climate change, including the value of human

health co-benefits and job creation, are substantial and should be considered in policy design.

Overall, green carbon occupies a complex position in climate mitigation strategies. While it cannot substitute for rapid reductions in fossil fuel emissions, it represents a critical complementary pathway that links climate goals with broader sustainability objectives (Marvin et al., 2023). Recognizing both the opportunities and limitations of green carbon is essential for designing policies and accounting frameworks that are environmentally credible, socially acceptable, and resilient over the long term. It is important to note that including passive CO₂ uptake in net anthropogenic emissions accounting may undermine climate goals (Allen et al., 2024).

Future Challenges and Research Gaps

Despite growing scientific and policy interest, significant uncertainties remain regarding the functioning, stability, and long-term climate relevance of green carbon (Munro et al., 2025). One of the most persistent challenges concerns the long-term persistence of soil carbon, particularly the extent to which management-induced carbon gains can be stabilized over decadal timescales. While recent advances have improved understanding of soil carbon fractions and stabilization mechanisms, the controls governing the formation and persistence of mineral-associated organic carbon under changing climatic conditions remain incompletely resolved.

Another major research gap lies in the interaction between biodiversity and green carbon sequestration. Although diverse plant communities are often associated with higher ecosystem resilience and productivity, the mechanisms through which biodiversity influences carbon inputs, microbial processing, and long-term stabilization are still poorly quantified. In many ecosystems, trade-offs may exist between maximizing carbon storage and achieving other ecological objectives, such as species conservation or water

regulation, underscoring the need for integrated, multifunctional assessments.

Compound and interacting climate stresses represent an additional and increasingly urgent challenge. Most existing studies examine the effects of single drivers, such as warming or altered precipitation, in isolation. However, real-world ecosystems are simultaneously exposed to multiple stressors, including heat extremes, drought, altered disturbance regimes, and nutrient limitations. Understanding how green carbon pools respond to these interacting pressures and whether thresholds or tipping points emerge remains a critical research frontier.

From a methodological perspective, there is a pressing need to improve the scaling of observations across space and time. Field measurements provide essential process-level insights but are inherently limited in spatial extent, while remote sensing products and models often lack sufficient resolution or mechanistic detail to capture belowground dynamics. Bridging these gaps will require long-term monitoring networks, standardized measurement protocols, and tighter integration between empirical data and modelling frameworks (Ciais et al., 2014).

Equally important are the socio-economic dimensions of green carbon dynamics. Land-use decisions are driven not only by biophysical constraints but also by economic incentives, governance structures, and cultural practices. Future research must therefore address how carbon-oriented management strategies interact with food security, rural livelihoods, and equity considerations. Without such integration, carbon sequestration efforts risk being short lived or socially unsustainable.

Finally, advancing green carbon science will depend on stronger interdisciplinary collaboration. Progress requires the combined expertise of soil scientists, ecologists, climatologists,

economists, and policy researchers. Bridging disciplinary boundaries is essential not only for improving scientific understanding, but also for translating green carbon knowledge into robust management practices and credible climate policies.

Conclusions

Green carbon constitutes a central, yet inherently vulnerable, component of the Earth's carbon system. Terrestrial ecosystems currently store vast quantities of carbon in vegetation, dead organic matter, and soils, and they play a decisive role in regulating atmospheric carbon dioxide concentrations. When protected and managed appropriately, these systems can contribute meaningfully to climate change mitigation while simultaneously supporting soil health, biodiversity, and a wide range of ecosystem services.

At the same time, green carbon cannot be regarded as a fixed or guaranteed solution. Its storage is dynamic and reversible, shaped by biological processes, environmental conditions, and human activities. Carbon accumulated over long periods can be rapidly lost through land-use change, disturbance, or climate extremes, often at rates that far exceed subsequent recovery. This asymmetry underscores the importance of prevention maintaining intact, high-carbon ecosystems over reliance on post-disturbance restoration alone.

The synthesis presented in this chapter highlights that effective green carbon management depends on recognizing ecosystem specific carbon dynamics, dominant carbon pools, and vulnerability to disturbance. Strategies that enhance carbon inputs, promote stabilization in long lived pools, and reduce disturbance pressures are most effective when tailored to local ecological and socio-economic contexts. In particular, the protection of existing high-carbon systems, such as forests and peatlands, emerges as one of the most robust and cost-effective climate mitigation options.

From a policy perspective, green carbon should be viewed as a complementary pathway rather than a substitute for rapid reductions in fossil fuel emissions. Its integration into climate strategies and carbon accounting frameworks requires credible monitoring systems, long-term commitments, and explicit acknowledgment of uncertainty, permanence, and potential trade offs. Aligning carbon sequestration objectives with food security, livelihoods, and ecosystem integrity remains a critical challenge.

Ultimately, green carbon offers a unique opportunity to connect climate mitigation with broader sustainability goals. Realizing this potential will require continued advances in scientific understanding, interdisciplinary collaboration, and governance approaches that balance ambition with realism. Recognizing both the promise and the limits of green carbon is essential for developing resilient, evidence-based pathways toward long-term climate stability.

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CHAPTER 3

THE RELATIONSHIP BETWEEN CLIMATE CHANGE, LIGHTNING AND THUNDERSTORMS

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Introduction

The interaction between climate change, lightning, and thunderstorms constitutes an important area of research for understanding the effects of global warming on atmospheric processes. Recent studies have demonstrated that rising global temperatures are substantially altering both large-scale atmospheric circulation systems and local weather conditions. As a consequence of these changes, an increasing trend has been observed in both the frequency and intensity of thunderstorms, accompanied by a parallel rise in lightning activity. This process is primarily associated with the long-term warming effect induced by anthropogenic greenhouse gas emissions in the atmosphere (Michibata, 2024; PARC, 2025).

The impacts of these changes extend beyond meteorological processes, producing direct and indirect consequences for ecosystems, human health, and infrastructure systems. Therefore, effective adaptation to climate change requires the development of comprehensive strategies that integrate multiple disciplines rather than relying on isolated approaches. Scientific evidence indicates that atmospheric warming enhances evaporation rates and increases atmospheric moisture content. The resulting increase in moisture and available energy creates more favorable conditions for thunderstorm development, leading to increases in both storm frequency and severity (Al-Saady, 2022).

Particularly throughout the twenty-first century, a substantial increase in thunderstorm activity is anticipated in regions experiencing elevated atmospheric humidity. This trend is directly linked to a corresponding increase in lightning occurrence. Global-scale projections suggest that lightning frequency could increase by as much as 30% under warmer climatic conditions. In addition, significant shifts in the geographical distribution of thunderstorms

have been observed. Regions previously considered relatively low risk are increasingly becoming areas of heightened vulnerability, thereby rendering disaster management and preparedness efforts more complex and less predictable (Collins et al., 2024).

Thunderstorms are among the principal mechanisms responsible for the global redistribution of water, playing a critical role in regulating both shortwave (solar) and longwave (terrestrial infrared) energy fluxes. In this context, water serves as a fundamental mediator influencing both short-term weather phenomena and long-term climate equilibria.

The climatic significance of lightning is closely linked to cloud electrification and storm dynamics. In electrically charged convective systems, the buoyancy force responsible for vertical cloud motion can arise from temperature differences of only approximately 1 °C. Temperature variations of this magnitude substantially influence charge separation and electrical discharge processes within clouds, rendering the physical mechanisms governing lightning formation highly sensitive. This sensitivity makes lightning a valuable indicator in climate change research, as global warming itself produces temperature changes of a comparable scale (Williams, 2005).

Lightning is a significant atmospheric phenomenon that occurs as a natural consequence of thunderstorms and is characterized by a complex interaction with climate change. Rising atmospheric temperatures not only increase the frequency of lightning occurrence but may also enhance the energy and intensity of individual lightning events. Scientific evidence suggests that each 1 °C increase in global temperature may result in an approximately 12% increase in lightning frequency (Price, 2009).

This trend has important environmental implications, particularly through the increased risk of wildfire ignition, which can

cause extensive ecosystem degradation. Furthermore, lightning contributes to the formation of ground-level ozone, thereby participating in processes that adversely affect air quality and pose additional risks to public health (Holzworth et al., 2021).

Lightning and Thunder

Lightning

Lightning is an electrical discharge that occurs within a cloud, between clouds, or between a cloud and the Earth's surface (Rakov & Uman, 2003). This discharge releases a substantial amount of energy and is observed as a flash of light. Lightning results from the accumulation and sudden discharge of electrical charges in the atmosphere. In particular, convective systems in the upper troposphere, characterized by variations in moisture and temperature, provide favorable conditions for lightning formation (Williams, 1992).

Lightning is generally classified into two primary types: cloud-to-ground (CG) and cloud-to-cloud (CC) lightning. Cloud-to-ground lightning occurs when an electrical current is discharged between a cloud and the Earth's surface and is generally considered the more destructive form due to its direct impacts on terrestrial environments, ecosystems, and infrastructure. In contrast, cloud-to-cloud lightning occurs between electrically charged regions within or between clouds and represents the most common form of lightning observed in nature. Studies indicate that lightning flashes occurring within or between clouds are approximately 5–10 times more frequent than cloud-to-ground flashes (NSSL, 2025)

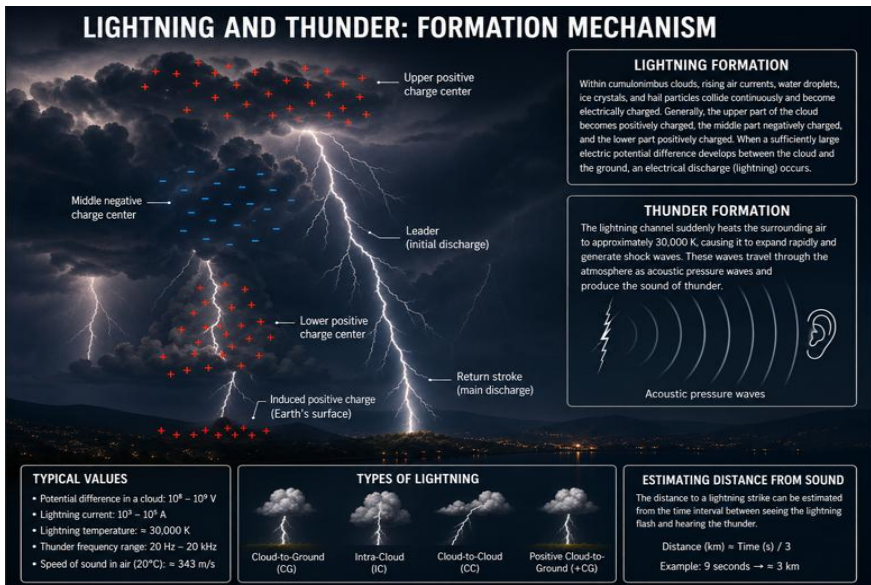


Figure 1. Mechanism of lightning and thunder formation

Causes of Lightning Formation

Lightning occurs as a result of imbalances in electrical charges within clouds. Generally, negative charges accumulating in the lower part of clouds interact with positive charges on the Earth's surface, initiating a process of electrical discharge. This process manifests itself as a sudden visible flash of light. A single lightning event often consists of multiple successive electrical discharges, and short-term variations in electric potential occur at the Earth's surface throughout this process (Price and Rind, 1994).

Statistical Trends in Lightning Frequency

According to long-term climate studies, lightning activity is closely associated with climate change. Warmer atmospheric conditions tend to increase the frequency of lightning occurrence. Research indicates that each 1°C increase in global temperature may lead to an approximately 12% increase in lightning activity (Price, 2009).

Despite limitations in the collection of historical lightning data, a significant increase has been observed in recent years, particularly in regions such as the Arctic that were previously characterized by low lightning activity. This increase is considered an important finding supporting the impact of anthropogenic climate change on atmospheric processes (Price and Rind, 1994).

Climate Change and Lightning Activity

The relationship between climate change and lightning is an important area of research in atmospheric sciences. Climate models predict that a warmer atmosphere will increase both the frequency of thunderstorms and their capacity to produce lightning (Price and Rind, 1994).

Some studies suggest that the total number of storms may decrease in the future. However, despite a potential decline in their overall number, the storms that do develop are expected to be more intense. Consequently, lightning occurrences may become more frequent, with estimates suggesting an increase of up to 30% on a global scale. This highlights more clearly the extent to which factors such as atmospheric instability and atmospheric moisture content influence lightning formation (Price, 2009).

Thunder

Thunder is a sound wave produced as a result of lightning heating the air to extremely high temperatures. Lightning heats the surrounding air to approximately 30,000 °C within seconds, causing the air to expand rapidly and generate shock waves (Uman, 2001). Thunder is a consequence of lightning and is not itself a direct electrical phenomenon.

Thunderstorms are meteorological phenomena that are particularly common in the Midwest region of the United States. These storms generally develop during late spring, summer, and

early autumn. The period of greatest activity occurs between May and July. During this time, warm and humid tropical air masses encounter cooler and drier polar air masses, creating favourable atmospheric conditions for storm development. However, if the atmosphere is sufficiently unstable and moist and a lifting mechanism is present, thunderstorms can occur at any time of the year (Center, 2000).

Thunderstorm Life Cycle

A thunderstorm generally begins with the formation of small cumulus clouds. As warm air near the Earth's surface rises, these clouds grow and first reach a more developed stage known as the cumulus congestus stage, before eventually evolving into cumulonimbus clouds, which are the true storm clouds. During this process, the heat released as water vapor condenses into droplets effectively fuels the cloud, promoting further growth and generating strong updrafts within it (Center, 2001).

When the storm reaches the mature stage, downdrafts begin to develop. These air currents lead to the onset of heavy precipitation and, in some cases, the formation of strong winds. As time progresses, the downward motions become dominant, causing the updrafts to weaken and preventing them from sustaining the storm. Consequently, the system loses its strength and gradually enters the dissipation stage (Figure 2).

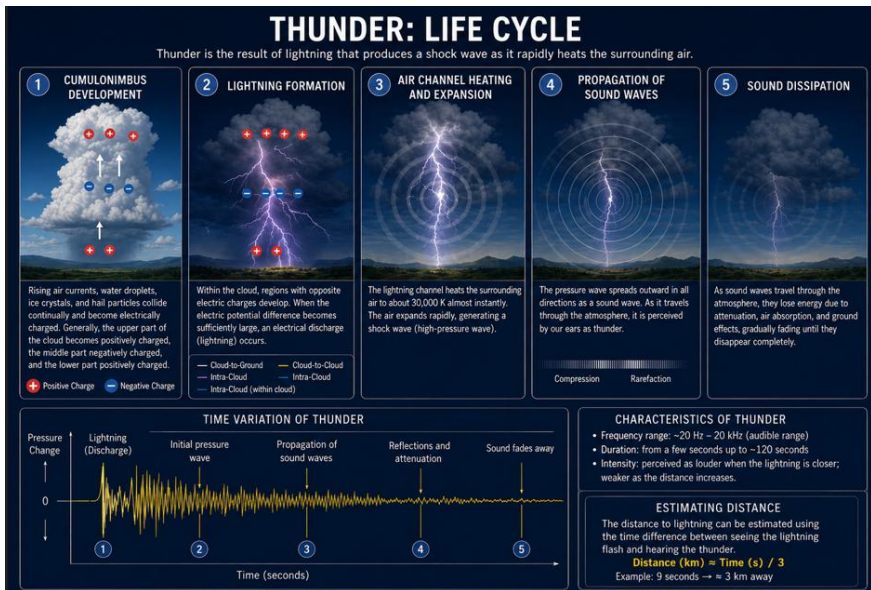


Figure 2. Thunderstorm Life Cycle

Types of Thunderstorms

Thunderstorms are classified according to their structural characteristics; Single-cell storms: These are short-lived storms (approximately 1 hour). They typically develop during summer afternoons as a result of surface heating and produce moderate precipitation, wind, lightning, and thunder (Center, 2000). Supercells: These are more organized and longer-lived storms. They contain a rotating updraft (mesocyclone) and are frequently associated with tornado formation (Center, 2000). Multicell storms: These storms develop when multiple storm cells trigger one another. Although each individual cell is short-lived (30–60 minutes), the system can persist for several hours and may produce hail, severe winds, and short-lived tornadoes (Center, 2000).

Effects on Thunderstorm Activity

As the climate warms, a clear and strong relationship has been observed between increasing temperatures and the intensity of thunderstorms. The general scientific consensus is that rising

temperatures make storms more energetic and intense, thereby creating conditions favourable for more severe precipitation events. Climate projections for the 21st century indicate that thunderstorm frequency is likely to increase, particularly in regions experiencing higher atmospheric moisture levels, and that lightning occurrences will become more frequent as a result (Rädler et al., 2019)

Storm Environments and Climate Change

Thunderstorms are highly sensitive to regional and seasonal variations in factors such as wind direction, wind intensity, and the distribution of atmospheric moisture. These balances are expected to change because of climate change. In particular, the number of days characterized by severe storm conditions is projected to increase toward the end of the 21st century, especially in the southern and eastern regions of the United States (Piper et al., 2019).

Climate models suggest that the atmospheric conditions responsible for triggering severe storms may occur more frequently in the future. Consequently, not only the number of storms but also their impacts are expected to become increasingly significant. Such storms can produce highly destructive and damaging weather phenomena, including severe winds, hail, and even tornadoes (Trapp et al., 2007).

Climate Change and Thunderstorms

Rising global temperatures significantly affect both the frequency and intensity of thunderstorms, as well as their spatial distribution. As greenhouse gas emissions increase, the atmosphere becomes warmer, strengthening the conditions that support storm development and creating more favorable environments for the occurrence of such events.

Climate Impacts and Atmospheric Consequences

Climate Change

Climate change is substantially altering long-term atmospheric weather patterns and, as a consequence, increasing both the frequency and intensity of extreme weather events such as thunderstorms and lightning. Human activities are largely responsible for this process. In particular, the extensive use of fossil fuels has increased greenhouse gas emissions, making it one of the primary causes of the global temperature rise observed over the past century (PARC, 2025).

Relationship Between Lightning, Thunder, and Climate

Lightning and thunder are closely associated with climate parameters such as atmospheric temperature, humidity, and convective instability (Romps et al., 2014). High surface temperatures and increased atmospheric moisture enhance atmospheric instability, thereby increasing the frequency of lightning occurrences (Price and Rind, 1994). Therefore, lightning activity is expected to increase as a consequence of global warming (Figure 3).

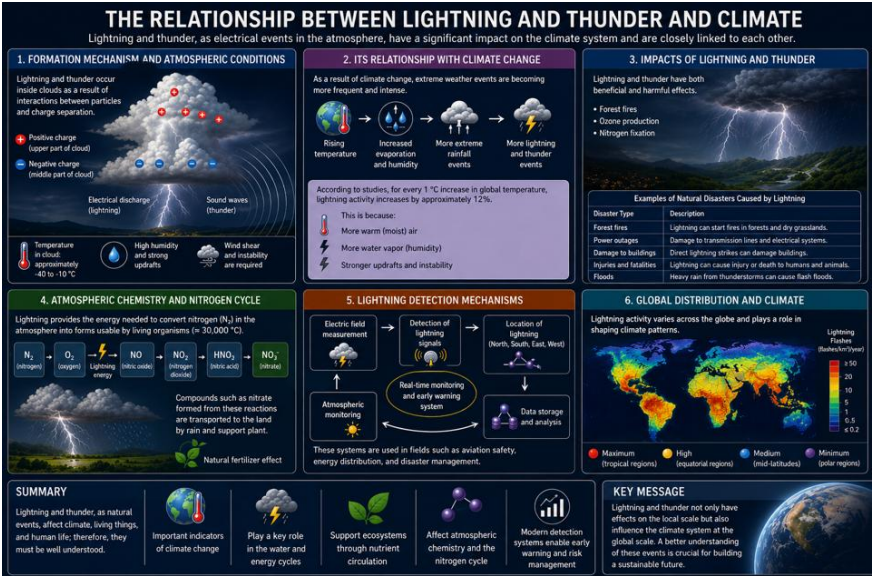


Figure 3. Relationship Between Lightning, Thunder, and Climate

Effects of Lightning on the Climate System

Lightning produces nitrogen oxides (NO_x), which contribute to the formation of tropospheric ozone (Schumann & Huntrieser, 2007). Ozone in the troposphere is formed by photochemical reactions involving sunlight and ozone precursor gases including nitrogen oxides (NO_x) and methane, carbon monoxide (CO), and non-methane volatile organic compounds (NMVOC; (Kinney, 2018; Jacob, 2000). These precursors originate from natural sources including wetland methane emissions, wildfires, biogenic hydrocarbon emissions, lightning NO_x, and NO_x emitted from soils, but also anthropogenic activities like fossil fuel combustion in power plants, industrial activities, transportation, or crop burning (Dewan&Lakhani, 2022). However, because the concentrations of tropospheric CO₂ and water vapor are much higher than those of ozone, the contribution of lightning to global warming is limited; its effects are more pronounced at the regional scale (Schumann & Huntrieser, 2007).

Regional and Global Distribution

Regional Distribution of Lightning

Lightning activity exhibits significant regional variations. For example, Florida is known as the “lightning capital” of the United States and is among the regions with the highest lightning density. The Gulf Coast, the Southeast, and the Great Plains are also characterized by high levels of lightning activity (Figure 4). In contrast, regions such as the Northern Great Plains have experienced increases in severe storms and hail events, which have posed significant challenges, particularly for agricultural activities (Ingram et al., 2013).

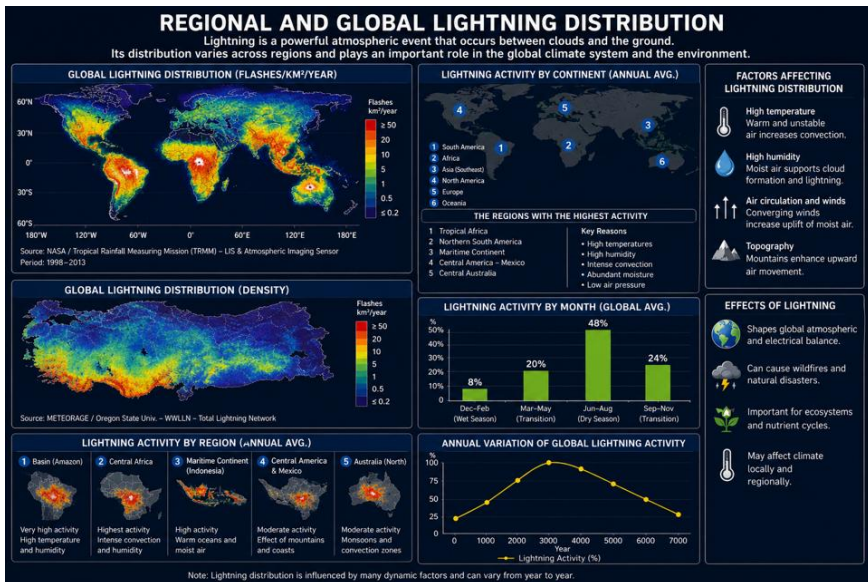


Figure 4. Global lightning distribution

Global Perspective on Lightning Activity

Studies conducted on a global scale indicate that lightning activity is particularly concentrated around the equatorial region. In this respect, the African continent stands out as a major center of lightning activity. In addition, Lake Maracaibo in Venezuela is recognized as one of the regions with the highest lightning density in the world. This demonstrates that geographical features can exert a strong influence on local weather phenomena (Albrecht et al., 2016).

Adaptation and Strategies

Regional Adaptation and Strategies

The effects of climate change are not experienced uniformly across all regions; rather, they vary spatially depending on atmospheric circulation patterns and regional climatic conditions. For example, the Midwestern United States is highly susceptible to

thunderstorms due to the frequent interaction of warm, moist air masses from the Gulf of Mexico with cooler continental air masses. This synoptic-scale variability enhances convective activity, which can directly impact agriculture, infrastructure, and socio-economic systems (Brooks et al., 2014).

For this reason, adapting to climate change has become more important than ever, particularly for more vulnerable communities. The development of adaptation strategies should not focus solely on reducing physical risks; it should also incorporate solutions aimed at protecting human health and social well-being (PARC, 2025).

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CHAPTER 4

PHYTOCHEMICALS IN FRUITS AND VEGETABLES WITH EMPHASIS ON GRAPES (*Vitis vinifera* L.)

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Introduction

As secondary metabolites produced by plants, phytochemicals fulfill essential roles in growth, reproduction, and defense against environmental stressors. The term “phytochemical” is derived from the Greek word *phyton*, meaning plant. These compounds contribute to the sensory properties of plant-based foods, including color, taste, and aroma, and are also associated with a wide range of biological activities relevant to human health (Naczki and Shahidi, 2004; Liu, 2013).

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Over the past decades, phytochemicals have become a major focus of nutrition research because of their potential health benefits. Epidemiological studies consistently indicate that diets rich in fruits and vegetables are associated with a reduced risk of chronic diseases, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders (Liu, 2013; Boeing et al., 2012). These protective effects are largely attributed to the presence of bioactive phytochemicals, which modulate oxidative stress, inflammation, and metabolic pathways.

Phytochemicals comprise a highly diverse and heterogeneous group of compounds. More than 8,000 phytochemicals have been identified to date, and this number continues to grow due to structural diversity arising from biochemical modifications such as hydroxylation, methylation, glycosylation, and acylation (Shahidi and Naczki, 2003).

Traditionally, phytochemicals are classified into five major groups:

- Carotenoids
- Alkaloids
- Nitrogen-containing compounds
- Organosulfur compounds
- Phenolic compounds

Among these, phenolic compounds represent the most extensively studied group due to their strong antioxidant properties and widespread occurrence in plant-based foods.

Fruits and vegetables are the primary dietary sources of phytochemicals. Phytochemicals such as phenolic acids, flavonoids, anthocyanins, lycopene, and sulfur-containing compounds, together

with essential micronutrients such as vitamins (A, B, C) and tocopherols, contribute significantly to their nutritional and functional value. These compounds not only provide essential nutrients but also exert protective biological effects through various molecular mechanisms.

Historically, phytochemicals have formed the foundation of traditional medicine systems, including Chinese, Indian (Ayurvedic), Egyptian, and Greek medicine, where plant-derived materials such as seeds, leaves, fruits, and roots have long been utilized for therapeutic purposes. This traditional knowledge has progressively evolved into modern concepts such as functional foods and nutraceuticals as scientific interest in plant bioactive compounds grew (Pan et al., 2013; Liu, 2013).

Despite extensive research, the precise molecular mechanisms underlying the beneficial effects of many phytochemicals remain incompletely understood. Ongoing studies continue to explore their bioavailability, metabolism, and interactions within biological systems.

Among phytochemical-rich foods, grapes (*Vitis vinifera* L.) are a particularly important source because of their high content of polyphenolic compounds. Grapes and grape-derived products, including wine, juice, and extracts, have been widely studied for their health-promoting properties. The unique phytochemical composition of grapes, especially their polyphenol profile, makes them a valuable model for studying the role of dietary phytochemicals in human health (Teissedre and Waterhouse, 2000).

Phytochemicals in Fruits and Vegetables

Fruits and vegetables are rich sources of structurally diverse phytochemicals that contribute to their nutritional quality and health-promoting properties. These bioactive compounds function as

antioxidants, anti-inflammatory agents, and modulators of metabolic pathways.

Classification of Phytochemicals

Phytochemicals represent a structurally diverse group of bioactive compounds synthesized through various plant metabolic pathways. Their classification is primarily based on chemical structure, biosynthetic origin, and functional properties. This classification is essential for understanding their biological roles, dietary significance, and potential applications in functional foods and nutraceuticals.

From a biochemical perspective, phytochemicals are generally divided into major groups including phenolic compounds, carotenoids, alkaloids, organosulfur compounds, and nitrogen-containing compounds. Each class encompasses a wide range of molecules with distinct chemical properties and biological activities. For instance, phenolic compounds are characterized by one or more hydroxyl groups attached to aromatic rings, whereas carotenoids are lipophilic isoprenoid derivatives responsible for pigmentation in many fruits and vegetables (Shahidi and Ambigaipalan, 2015).

Phenolic compounds constitute the most abundant and widely studied class of phytochemicals. They are subdivided into flavonoids (e.g., flavonols, flavan-3-ols, anthocyanins), phenolic acids, tannins, and stilbenes. These compounds are largely derived from the phenylpropanoid pathway and are known for their strong antioxidant capacity and ability to modulate cellular signaling pathways (Manach et al., 2004). In contrast, carotenoids such as β -carotene, lycopene, and lutein are synthesized via the isoprenoid pathway and contribute to photoprotection and vision (Rao and Rao, 2007).

Table 1. Major Classes of Phytochemicals in Fruits and Vegetables

Class	Examples	Major Sources	Biological Functions	Reference
Phenolics	Flavonoids, phenolic acids, tannins, stilbenes	Fruits, vegetables, tea, wine	Antioxidant, anti-inflammatory, cardioprotective	Manach et al., 2004
Carotenoids	β -carotene, lycopene, lutein	Carrots, tomatoes, leafy greens	Antioxidant, vision protection, immune support	Rao and Rao, 2007
Alkaloids	Caffeine, capsaicin, morphine	Coffee, cocoa, chili peppers	Neuroactive effects, analgesic, stimulant	Aniszewski, 2015
Organosulfur compounds	Allicin, glucosinolates, sulforaphane	Garlic, onion, broccoli	Detoxification, anticancer activity	Traka and Mithen, 2009
Nitrogen-containing compounds	Cyanogenic glycosides, amines, glucosinolates	Legumes, Rosaceae species (e.g., bitter almonds), Brassicaceae	Plant defense (herbivore deterrence), nitrogen storage, metabolic regulation	Crozier et al., 2006

Alkaloids, another important class, are nitrogen-containing heterocyclic compounds often associated with diverse pharmacological effects. Many alkaloids, including caffeine and capsaicin, exhibit potent physiological activities by interacting with various receptors in the nervous system (Aniszewski, 2015). Organosulfur compounds, commonly found in *Allium* and cruciferous vegetables, are particularly recognized for their role in detoxification and cancer prevention, largely through modulation of phase II detoxifying enzymes (Traka and Mithen, 2009).

Phytochemicals rarely act in isolation within plant matrices. Instead, they exist as complex mixtures that may exhibit synergistic or antagonistic interactions. This complexity is why a useful classification must account for both chemical diversity and biological function.

The classification of phytochemicals provides a basis for understanding their nutritional and therapeutic potential. However, due to the continuous discovery of new compounds and the structural complexity resulting from various biochemical modifications, this classification remains dynamic and subject to ongoing refinement. Future research combining metabolomics and systems biology should clarify the diversity and function of phytochemicals.

Biological Activities of Phytochemicals

Phytochemicals exert a wide spectrum of biological activities that contribute significantly to the prevention and management of chronic diseases. These bioactive compounds influence multiple cellular and molecular pathways, often acting through antioxidant, anti-inflammatory, anticancer, cardioprotective, and neuroprotective mechanisms. These effects involve complex interactions across multiple biological pathways rather than a single one.

Antioxidant Activity

One of the most extensively studied biological functions of phytochemicals is their antioxidant capacity. Reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, and hydrogen peroxide, are generated as by-products of normal cellular metabolism. Excessive ROS production leads to oxidative stress, which is implicated in the pathogenesis of numerous chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Lobo et al., 2010).

Phytochemicals, particularly polyphenols, act as potent antioxidants through multiple mechanisms:

- Direct scavenging of free radicals
- Chelation of pro-oxidant metal ions (e.g., Fe^{2+} , Cu^{2+})
- Upregulation of endogenous antioxidant defense systems

Compounds such as flavonoids and phenolic acids can donate hydrogen atoms or electrons to neutralize free radicals, thereby interrupting oxidative chain reactions (Pisoschi and Pop, 2015). Additionally, certain phytochemicals activate transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2), leading to increased expression of antioxidant enzymes.

Anti-Inflammatory Effects

Chronic inflammation is a central factor in the development of metabolic syndrome, cancer, and cardiovascular diseases. Phytochemicals modulate inflammatory responses by influencing key signaling pathways, particularly nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinases (MAPKs) (González et al., 2011).

Polyphenols such as quercetin, resveratrol, and catechins have been shown to:

- Inhibit the activation of NF- κ B
- Reduce the production of pro-inflammatory cytokines (e.g., TNF- α , IL-6, IL-1 β)
- Suppress cyclooxygenase (COX) and lipoxygenase (LOX) enzyme activity

Together, these mechanisms attenuate chronic inflammatory responses and may lower the risk of inflammation-related diseases.

Anticancer Activity

Phytochemicals have been extensively investigated for their potential role in cancer prevention and therapy. Their anticancer effects are mediated through various mechanisms, including:

- Induction of apoptosis (programmed cell death)
- Inhibition of cell proliferation
- Suppression of angiogenesis and metastasis
- Modulation of cell signaling pathways (e.g., PI3K/Akt, MAPK)

For example, flavonoids and stilbenes can regulate the expression of tumor suppressor genes and oncogenes, thereby influencing cancer cell survival and progression (Pan et al., 2010). Additionally, phytochemicals may enhance the effectiveness of conventional chemotherapeutic agents while reducing their toxicity.

Cardioprotective Effects

Phytochemicals help maintain cardiovascular health by targeting several risk factors for cardiovascular disease. These

compounds improve vascular function, lipid metabolism, and oxidative balance.

Key cardioprotective mechanisms include:

- Improvement of endothelial function through increased nitric oxide (NO) bioavailability
- Reduction of low-density lipoprotein (LDL) oxidation
- Inhibition of platelet aggregation
- Modulation of lipid profiles

Epidemiological studies have shown that diets rich in polyphenols are associated with reduced incidence of hypertension, atherosclerosis, and coronary heart disease (Del Rio et al., 2013).

Neuroprotective Effects

Neurodegenerative diseases such as Alzheimer's and Parkinson's disease are closely linked to oxidative stress, inflammation, and protein aggregation. Phytochemicals have demonstrated neuroprotective potential through several mechanisms:

- Reduction of oxidative stress in neuronal cells
- Inhibition of amyloid-beta aggregation
- Modulation of neuronal signaling pathways
- Enhancement of synaptic plasticity

Flavonoids, in particular, have been shown to cross the blood–brain barrier and exert protective effects on neuronal function (Spencer, 2008).

Antimicrobial and Metabolic Effects

In addition to the major biological activities described above, phytochemicals also exhibit antimicrobial and metabolic regulatory effects. Many plant-derived compounds possess antibacterial, antiviral, and antifungal properties that aid plant defense and may benefit human health.

Furthermore, phytochemicals influence metabolic processes by regulating glucose metabolism, improving insulin sensitivity, and modulating lipid metabolism; these effects are particularly relevant in the prevention and management of metabolic disorders such as obesity and type 2 diabetes (Hanhineva et al., 2010).

The biological activities of phytochemicals are varied and interconnected. Because they act on multiple molecular targets at once, they are strong candidates for functional foods, nutraceuticals, and therapeutic agents. However, further research is needed to fully elucidate their mechanisms of action, bioavailability, and long-term effects in human populations.

Phytochemical Composition of Grapes (*Vitis vinifera* L.)

Grapes (*Vitis vinifera* L.) are among the most extensively studied fruit crops due to their rich and diverse phytochemical composition. These compounds are primarily secondary metabolites synthesized by plants in response to environmental stimuli and physiological processes. The phytochemical profile of grapes is highly complex and includes a wide range of polyphenols, carotenoids, organic acids, and other bioactive compounds that contribute to both plant physiology and human health benefits.

Overview of Grape Phytochemicals

The phytochemical composition of grapes is dominated by polyphenolic compounds, which represent the most abundant and

biologically active constituents. These include flavonoids (flavonols, flavan-3-ols, and anthocyanins), non-flavonoid phenolics (phenolic acids and stilbenes), and tannins. In addition to polyphenols, grapes also contain carotenoids, vitamins, and minor quantities of alkaloids and nitrogen-containing compounds (Flamini et al., 2013).

Polyphenols are responsible for many of the sensory characteristics of grapes, such as color, bitterness, and astringency, as well as their antioxidant properties. The concentration and composition of these compounds vary significantly depending on grape cultivar, maturity stage, environmental conditions, and post-harvest handling (Teixeira et al., 2013).

Biosynthetic Pathways of Grape Phytochemicals

Grape phytochemicals are synthesized through interconnected metabolic pathways, primarily:

- Phenylpropanoid pathway
- Shikimate pathway
- Isoprenoid pathway

The phenylpropanoid pathway is the principal pathway for the biosynthesis of most grape polyphenols, including flavonoids, stilbenes, and phenolic acids. This pathway originates from phenylalanine, which is converted into cinnamic acid by phenylalanine ammonia-lyase (PAL), a key regulatory enzyme (Ali et al., 2010).

Subsequent enzymatic reactions lead to the formation of diverse phenolic compounds:

- Flavonoids (via chalcone synthase and flavonoid biosynthetic enzymes)

- Anthocyanins (through glycosylation and methylation processes)
- Stilbenes (via stilbene synthase, particularly resveratrol)

The isoprenoid pathway contributes to the synthesis of carotenoids and other terpenoid compounds, which contribute to pigmentation and photoprotection.

Distribution of Phytochemicals in Grape Tissues

Phytochemicals are unevenly distributed across different grape tissues, with significant implications for their nutritional and functional properties.

- Grape skin: Rich in anthocyanins, flavonols, and stilbenes (especially resveratrol)
- Grape seeds: High concentrations of proanthocyanidins (condensed tannins) and catechins
- Grape pulp: Lower levels of phenolic compounds, primarily phenolic acids and simple sugars
- Grape stems: Contain tannins and various flavonoids

This distribution explains why grape-derived products such as red wine, which involve prolonged skin contact during fermentation, contain higher levels of polyphenols compared to white wine (Waterhouse et al., 2016).

Major Phytochemicals in Grapes

Grapes (*Vitis vinifera* L.) are particularly rich in polyphenolic compounds, which represent the most biologically active class of phytochemicals. These compounds are primarily synthesized via the phenylpropanoid pathway and are responsible for many of the health-promoting properties associated with grape consumption. The

major classes of phytochemicals in grapes include flavonoids, anthocyanins, stilbenes, proanthocyanidins, and phenolic acids.

Phenolic Compounds

Phenolic compounds constitute the largest group of phytochemicals in grapes and are characterized by the presence of one or more hydroxyl groups attached to aromatic rings. They are mainly divided into flavonoids and non-flavonoid phenolics.

Flavonoids

Flavonoids are the most abundant polyphenols in grapes and include several subclasses:

- Flavonols (e.g., quercetin, kaempferol)
- Flavan-3-ols (e.g., catechin, epicatechin)
- Anthocyanins
- Proanthocyanidins

Flavonoids constitute the largest group of polyphenols in grapes and are widely distributed in grape skins and seeds. Structurally, flavonoids are characterized by a C₆–C₃–C₆ backbone and are subdivided into several subclasses, including flavonols, flavan-3-ols, and anthocyanins.

Flavonols such as quercetin, kaempferol, and myricetin are primarily located in grape skins and contribute to UV protection in plants. Flavan-3-ols, including catechin and epicatechin, are abundant in grape seeds and are key precursors of proanthocyanidins. These compounds have strong antioxidant activity and help reduce oxidative stress and inflammation (He and Giusti, 2010; Teixeira et al., 2013).

These compounds are primarily synthesized through the phenylpropanoid pathway and function in plant pigmentation, UV protection, and defense.

Anthocyanins

Anthocyanins are water-soluble pigments responsible for the red, purple, and blue coloration of grape skins. These compounds are glycosylated derivatives of anthocyanidins and are mainly found in red grape varieties.

The most abundant anthocyanin in grapes is malvidin-3-*O*-glucoside, followed by delphinidin, petunidin, peonidin, and cyanidin derivatives. Anthocyanin composition is influenced by grape cultivar, environmental conditions, and ripening stage (Castillo-Muñoz et al., 2009).

Anthocyanins are known for their potent antioxidant properties and have been associated with improved cardiovascular health, neuroprotection, and anti-inflammatory effects (He and Giusti, 2010).

Stilbenes

Stilbenes are a class of phytoalexins synthesized by plants in response to stress conditions such as pathogen attack, UV radiation, and mechanical injury.

Resveratrol

Among stilbenes, resveratrol (3,5,4'-trihydroxystilbene) is the most extensively studied compound in grapes. Resveratrol is primarily found in grape skins and is present in higher concentrations in red grapes compared to white varieties. It exists in both *trans*- and *cis*-isomeric forms, with *trans*-resveratrol being more biologically active.

Resveratrol shows a wide range of biological activities:

- **Antioxidant effects:** Neutralizes reactive oxygen species (ROS)
- **Anti-inflammatory effects:** Inhibits pro-inflammatory mediators
- **Cardioprotective properties:** Improves endothelial function
- **Anticancer activity:** Induces apoptosis and inhibits tumor progression

This compound has been widely investigated for its cardioprotective, anticancer, anti-inflammatory, and anti-aging properties. It exerts its biological effects through modulation of multiple molecular pathways, including activation of sirtuins and inhibition of inflammatory signaling pathways (Baur and Sinclair, 2006).

Proanthocyanidins

Proanthocyanidins, also known as condensed tannins, are oligomeric and polymeric forms of flavan-3-ols. These compounds are predominantly found in grape seeds, although they are also present in skins and stems.

They are composed of catechin and epicatechin units and can form complex polymeric structures. Proanthocyanidins contribute to the astringency and bitterness of grapes and grape-derived products.

From a biological perspective, they are among the most potent antioxidants (often higher than vitamins C and E) in grapes and have demonstrated cardioprotective, anti-inflammatory, and antimicrobial properties (Cosme et al., 2018).

Phenolic Acids

Phenolic acids represent a smaller but significant group of grape phytochemicals. They are divided into two main categories:

- Hydroxybenzoic acids (e.g., gallic acid, protocatechuic acid)
- Hydroxycinnamic acids (e.g., caffeic acid, *p*-coumaric acid, ferulic acid)

These compounds are found in both free and bound forms and contribute to the antioxidant capacity, flavor, and stability of grape products. They also serve as precursors for more complex polyphenolic compounds (Flamini et al., 2013).

Summary of Major Grape Phytochemicals

Table 2. Major Phytochemicals in Grapes and Their Biological Activities

Compound Class	Key Compound s	Major Locatio n	Biological Effects	Referenc e
Flavonoids	Quercetin, catechin, epicatechin	Skin, seeds	Antioxidant, anti-inflammatory, cardioprotective	He and Giusti, 2010; Teixeira et al., 2013
Anthocyanins	Malvidin, delphinidin, cyanidin	Skin	Antioxidant, neuroprotective, cardioprotective	Castillo-Muñoz et al., 2009

Compound Class	Key Compound s	Major Location	Biological Effects	Reference
Stilbenes	Resveratrol	Skin	Anticancer, anti-inflammatory, cardioprotective	Baur and Sinclair, 2006
Proanthocyanidins	OPCs (catechin polymers)	Seeds	Strong antioxidant, cardioprotective	Cosme et al., 2018
Phenolic acids	Gallic acid, caffeic acid	Pulp, skin	Antioxidant, flavor stability	Flamini et al., 2013

Discussion

This review examines the potential of phytochemicals, particularly those derived from grapes (*Vitis vinifera* L.), in promoting human health through multiple biological mechanisms. Despite the extensive body of literature demonstrating antioxidant, anti-inflammatory, cardioprotective, anticancer, and neuroprotective effects, several critical limitations must be addressed to accurately interpret these findings and translate them into clinical and nutritional applications.

One of the most prominent challenges in phytochemical research is the discrepancy between *in vitro* efficacy and *in vivo* relevance. Many studies report strong biological activities using isolated compounds at concentrations far exceeding those achievable through dietary intake. For example, polyphenols such as resveratrol and quercetin demonstrate potent antioxidant and anticancer effects

in cell culture systems; however, their rapid metabolism and low systemic bioavailability significantly limit their physiological impact in humans (Manach et al., 2004; Del Rio et al., 2013). This raises important questions regarding the translational value of such findings and points to the need for more physiologically relevant experimental designs.

Another major limitation lies in the complex metabolism and bioavailability of phytochemicals. After ingestion, most grape polyphenols undergo extensive phase II metabolism, including glucuronidation, sulfation, and methylation, leading to the formation of metabolites with altered biological activity. Furthermore, the role of the gut microbiota in transforming phytochemicals into bioactive metabolites has gained increasing attention. Microbial-derived metabolites may exert distinct or even enhanced biological effects compared to their parent compounds, suggesting that individual variability in gut microbiota composition could significantly influence health outcomes (Cardona et al., 2013). This introduces an additional layer of complexity and highlights the importance of personalized nutrition approaches.

The matrix effect and synergistic interactions among phytochemicals represent another critical aspect often overlooked in reductionist research approaches. While many studies focus on isolated compounds such as resveratrol, whole grapes and grape-derived products contain complex mixtures of polyphenols, flavonoids, and other bioactives that may interact synergistically. Evidence suggests that these interactions can enhance antioxidant capacity and biological efficacy beyond what is observed with individual compounds alone (Liu, 2013). Therefore, future research should prioritize whole-food approaches and consider phytochemical interactions rather than focusing exclusively on single compounds.

Variability in phytochemical composition also poses a significant challenge for standardization and reproducibility. The concentration and profile of grape phytochemicals are influenced by numerous factors, including grape cultivar, geographic origin, climatic conditions, agricultural practices, ripening stage, and processing methods. This variability complicates comparisons across studies and limits the ability to establish consistent dietary recommendations or therapeutic dosages (Teixeira et al., 2013). Advanced analytical techniques such as metabolomics and high-resolution mass spectrometry may help address this issue by providing more comprehensive phytochemical profiling.

From a clinical perspective, human intervention studies remain limited and often inconclusive. Many trials involve small sample sizes, short durations, and heterogeneous study designs, making it difficult to draw definitive conclusions about the long-term health benefits of grape phytochemicals. Furthermore, differences in dosage, formulation (e.g., whole grapes vs. extracts), and participant characteristics contribute to inconsistent findings. There is a clear need for well-designed, large-scale, randomized controlled trials with standardized protocols to validate the health effects observed in preclinical studies (Del Rio et al., 2013).

Another emerging area of interest is the impact of food processing and storage on phytochemical stability and bioactivity. While processing techniques such as fermentation may enhance the availability of certain compounds, others may lead to degradation or transformation into less active forms. Similarly, storage conditions can significantly influence phytochemical retention. Understanding these factors is essential for optimizing the delivery of bioactive compounds through diet and functional food products.

Finally, the concept of phytochemicals as part of a systems biology framework is gaining traction. Rather than acting through

single targets, these compounds influence complex networks of signaling pathways, gene expression, and metabolic processes. This multi-targeted mode of action aligns with the multifactorial nature of chronic diseases and supports the integration of phytochemicals into preventive and therapeutic strategies.

In conclusion, while grape phytochemicals exhibit considerable promise as health-promoting agents, their effective application requires a more nuanced understanding of their bioavailability, metabolism, interactions, and variability. Future research should focus on bridging the gap between experimental findings and clinical relevance, incorporating advanced analytical techniques, standardized methodologies, and personalized nutrition approaches. Such efforts will be essential for fully realizing the potential of phytochemicals in improving human health.

Conclusion

Phytochemicals represent a diverse and biologically active group of compounds that contribute substantially to the health-promoting properties of fruits and vegetables. Among these, grapes (*Vitis vinifera* L.) are an exceptionally rich source of polyphenolic compounds, including flavonoids, anthocyanins, stilbenes, and proanthocyanidins.

The evidence reviewed here shows the wide-ranging biological activities of grape phytochemicals, particularly their antioxidant, anti-inflammatory, cardioprotective, anticancer, and neuroprotective effects. These properties make grapes and grape-derived products useful components of a health-promoting diet and good candidates for functional foods and nutraceuticals.

However, significant challenges remain, particularly regarding bioavailability, variability in phytochemical composition, and the limited availability of robust clinical data. Addressing these

challenges will be essential for translating experimental findings into practical dietary recommendations and therapeutic applications.

Future research should focus on:

- Conducting well-designed human clinical trials
- Investigating phytochemical interactions and synergistic effects
- Exploring the role of gut microbiota
- Developing advanced processing and delivery systems to enhance bioavailability

A deeper understanding of grape phytochemicals will advance nutritional science and support evidence-based strategies for disease prevention and health promotion.

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CHAPTER 5

PLANT BIOSTIMULANTS: CONFUSIONS AND EFFORTS TO RESOLVE THEM

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İBRAHİM BOLAT²

Introduction

In sustainable horticulture today, biostimulants are widely used in the form of seaweed extracts, humic and fulvic agents, protein hydrolysates, amino acids, arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), silicon-based compounds, and various biological extracts (du Jardin, 2025b). These products are among the important components of sustainable production systems because they increase the efficiency of plant nutrient utilization, improve tolerance to abiotic stress conditions, and enhance product quality. In particular, it has been reported that biostimulant applications in fruit, viticulture, and vegetable cultivation support plant growth and development, increase the efficiency of nutrient uptake and utilization, improve plant

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performance under environmental stress conditions, and have positive effects on product quality (Basile et al., 2020; Andreotti et al., 2022).

Citrus fruits (*Citrus spp.*) are among the perennial fruit species sensitive to drought, salinity, high temperatures, and calcareous soil conditions. Therefore, biostimulant applications are considered an important management tool in citrus cultivation to promote root development, increase nutrient uptake, and reduce the negative effects of abiotic stresses. In particular, mycorrhizal fungi and rhizobacteria increase the uptake of various nutrients, especially phosphorus, by expanding the root surface area, improve water use efficiency, and mitigate the effects of drought and salinity stress (Atawia et al., 1997).

Biostimulants are widely used not only in citrus fruits but also in the cultivation of many economically important horticultural crops such as apple (*Malus domestica*), cherry (*Prunus avium*), grape (*Vitis vinifera*), kiwi (*Actinidia deliciosa*), pomegranate (*Punica granatum*), and strawberry (*Fragaria* × *ananassa*). Research shows that biostimulant applications contribute to increased yield, improved tolerance to climate-induced stress conditions, enhanced fruit size and color development, increased vitamin and bioactive compound content, enhanced antioxidant capacity, and extended post-harvest storage and shelf life. In addition, biostimulants play an important role in improving crop quality by increasing photosynthetic efficiency, enhancing nutrient use efficiency, and regulating plant metabolism (Gonçalves et al., 2020, Frioni et al., 2018, Donno et al., 2013, Abubakar et al., 2013, Soppelsa et al., 2019). These products, which vary in category depending on the region, are defined as a new type of fertilizer. However, how biostimulants substantiate these claims is not fully explained. Different protocols established by various countries create bottlenecks in the definition of biostimulants. Therefore,

biostimulants are viewed with suspicion by the agricultural sector, especially growers, worldwide, leading to problems in their marketing and use.

The Concept of Plant Biostimulants

Humic acids derived from seaweed extracts have been known to be used in agriculture for decades, even centuries. What is new here is the concept of biostimulants. The word stimulant is, of course, a way of describing this context and this concept. The oldest article that uses the word biostimulant in its title and describes the use of organic biostimulants was published in 1991 in the *Journal of Sustainable Agriculture* by a group (Russo, R. O., & Berlyn, G. P.) affiliated with Yale University (Russo and Berlyn, 1991).

Researchers have described the use of organic biostimulants, primarily acids, seaweed extracts, vitamins, and a mixture thereof, to aid in low-input and sustainable agriculture. They reported that the amount of fertilizer given to the plant can be reduced with the help of biostimulants, and plant growth can be maintained. In their work over 30 years ago, Russo and Berlyn defined biostimulants as "non-fertilizer products which have a beneficial effect on plant growth." According to this definition, fertilizers are essentially and primarily nutrient providers, and the beneficial effects of biostimulants on plants stem not from nutrient provision but from other effects (Russo and Berlyn, 1991).

In their article published in the *International Lawn Society Research Journal*, pioneering researchers Zhang and Schmidt from Virginia Polytechnic Institute and State University defined biostimulants as "materials, other than fertilizers, that promote plant growth when they are applied in small quantities" (Zhang and Schmidt, 1997).

Today, we know that peptides and some amino acids have the capacity to chelate nutrients (e.g., by combining them). A. M.

Kinnersley, a pioneer in this field, reported in his 1993 article that biostimulants can act as phytochelates to facilitate the absorption of certain nutrients such as zinc, copper, and iron, and can also protect plants against some toxic components such as phytochelatins (PCs). Therefore, when we examine products supplemented with biostimulants, this study suggests that one of the mechanisms of action of biostimulants may be chelation. Furthermore, as mentioned in the abstract of the article, it has been reported that when growth is restricted due to metal excess or deficiency, plants respond by synthesizing non-protein-forming chelating agents. The researcher emphasized that biostimulants such as humic substances and lactic acid oligomers share common properties with both phytosiderophores and phytochelates (Kinnersley, 1993).

In their 2000 article, pioneering researchers Xunzhong Zhang and Schmidt from Virginia Polytechnic Institute and State University reported that the proposed function of these products was to provide hormones to plants, and they defined biostimulants as "hormone-containing products" (Zhang and Schmidt, 2000). This study aimed to determine whether the endogenous antioxidant concentration of two drought-affected grass species is sensitive to exogenous hormone-containing products (HCPs). Two-week-old longleaf grass (*Festuca arundinacea* Schreb.) and creeping grass (*Agrostis palustris* Huds. A.) seedlings were treated with two HCPs (326 g ha⁻¹ of seaweed extract (SWE) or 5 L ha⁻¹ of humic acid (HA, 25% active ingredient)) applied alone or in combination (Zhang and Schmidt, 2000).

Existing Legislations Around the World

To ensure the reliability of biostimulant products from farmers to end users, specific assurances regarding both their safety and efficacy must be provided. Indeed, in the study published in 1993, which we have just mentioned, Kinnersley points out that

inconsistent crop performance observed in the field is a significant factor limiting the application of biostimulants in agriculture and horticulture (Kinnersley, 1993). It appears that this situation remains valid even after nearly thirty years.

To clarify the scientific validity of the biostimulant concept, the European Commission requested a scientific review from the scientific community, and the results of this review were presented in a report in 2012. This report, prepared by Patrick Du Jardin, presented significant evidence regarding the capacity of these products to increase plant growth, stress tolerance, and nutrient use efficiency. The report also addressed how these products should be produced for the market (Du Jardin, 2012).

Canada regulates plant biostimulants as "supplement" under the CFIA Fertilizer Act. In the US, the industry lobby is known to be currently lobbying the USDA/EPA/AAPFCO for a definition. While the definition of biostimulants adopted in the US in March 2023 largely coincides with the European Union's definition, the phrase "biostimulants act to support the plant's natural nutritional processes" sets it apart slightly. Functionally, the difference between the two is as follows: In the European context, "biostimulants" target resistance to abiotic stress in an indirect way (chelating mechanisms, etc.). In the US definition, however, biostimulants, in addition to indirect effects, directly influence plant growth, increasing growth quality and yield. Another issue debated in the US is the definitional intersection of plant growth regulators (PGRs) and biostimulants. Plant growth regulators, and even naturally occurring plant hormones such as auxins, cytokines, and gibberellins, are currently considered pesticides by all legislative bodies globally when used at any concentration. Biostimulants, on the other hand, are positioned as elements that optimize plant nutrition processes but do not fall into the category of growth regulators such as auxins, cytokines, and gibberellins (Yakhin et al., 2017).

Mexico recognizes plant growth regulators (PGRs) under its fertilizer regulations. Brazil's 2023 update to the definition of biostimulants does not include microorganisms, but it does include biological fertilizers. Microorganisms not included in the definition of biological fertilizers form a category called inoculants and microbial soil conditioners. India's 2024 update included microorganisms within the definition of biostimulants, but did not include biological fertilizers. Toxicology data was excluded for specific product types, and specific characteristics were published. China recognized plant biostimulants in its 2021 fertilizer regulation update. In Australia, the definition of biostimulants is assessed on a case-by-case basis for each product, and definitions vary depending on the situation. In New Zealand, the definition of biostimulants under the ACVM Act is similar to that in Australia. Each product is evaluated individually, and definitions vary depending on the situation. Therefore, there are still significant challenges in homogenizing and standardizing the definition and concept of biostimulants on a global scale, but work in this area continues (Du Jardin et al., 2025a).

Plant Biostimulant in Regulations (Eu Regulation 2019/1009)

In the Regulation on the Provision of Fertilizing Products ('FPR'), plant biostimulants are defined as follows:

A plant biostimulant shall be an EU fertilising product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere:

- (a) nutrient use efficiency;
- (b) tolerance to abiotic stress;
- (c) quality traits; or

(d) availability of confined nutrients in the soil or rhizosphere.

An examination of the definition of biostimulants in European Union regulations reveals that they are defined by claims such as improving agricultural functions, increasing efficiency in nutrient use, resistance to abiotic stress, quality characteristics, or the availability of limited nutrients in the soil or rhizosphere. Therefore, it is crucial to understand that biostimulants are defined by claims related to their agricultural functions, not by their composition or mode of action, and this represents a significant difference with regard to plant protection products. Under European regulations, a detailed description of the product's mechanism of action is not mandatory for placing these products on the market.

Current Legislation in Türkiye

Türkiye's 2018 update does not recognize the term "biostimulant," but it does include the term "microbial fertilizers." According to the regulation on organic, mineral, and microbial fertilizers used in agriculture, published in the Official Gazette, microbial fertilizers are defined as follows:

These are products containing live microorganisms that play a role in providing essential substances for plant growth, increasing the availability of plant nutrients, and/or improving soil quality.

Reports from Different Organizations

The International Organization for Standardization (OECD) has established a common definition of biostimulants. According to this definition, a plant biostimulant is:

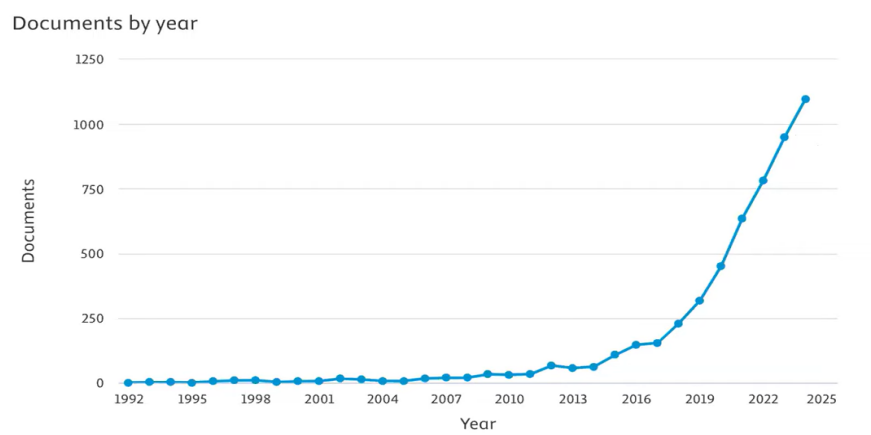
A biostimulant is a substance(s), microorganism(s), or mixture thereof that, when applied to seeds, plants, the rhizosphere, soil, or other growing media, supports the plant's natural nutritional process, regardless of the biostimulant's nutrient content. Plant

biostimulants thus improve nutrient availability, uptake or utilization efficiency, tolerance to abiotic stress, and consequently growth, development, quality, or yield (ISO/TC 134, 2022).

Biostimulants in Scientific Literature

Scientific literature shows a significant increase in the global adoption rate of biostimulants. In this context, studies containing both the terms "biostimulant" and "plant" were searched in the Scopus database based on titles and keywords, and the distribution of the resulting publications by year is given in Figure 1.

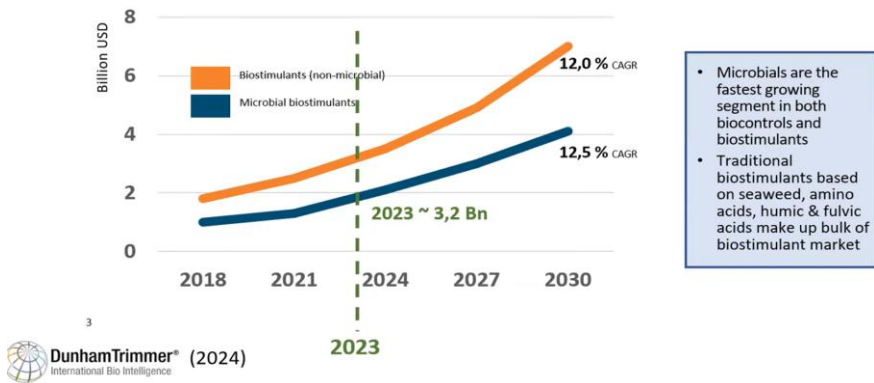
Figure 1: Studies containing the terms “biostimulant” and “plant” in the Scopus database



Source: Du Jardin et al., 2025a.

The success of stimulants is indicated not only by their widespread acceptance in the scientific literature, but also by the rapid growth of the market for biostimulant products. A study published in 2024 clearly shows that there is a growing market for both microbial and non-microbial biostimulants. The status of biostimulants in the global market is shown in Figure 2 (Dunham Trimer, 2024).

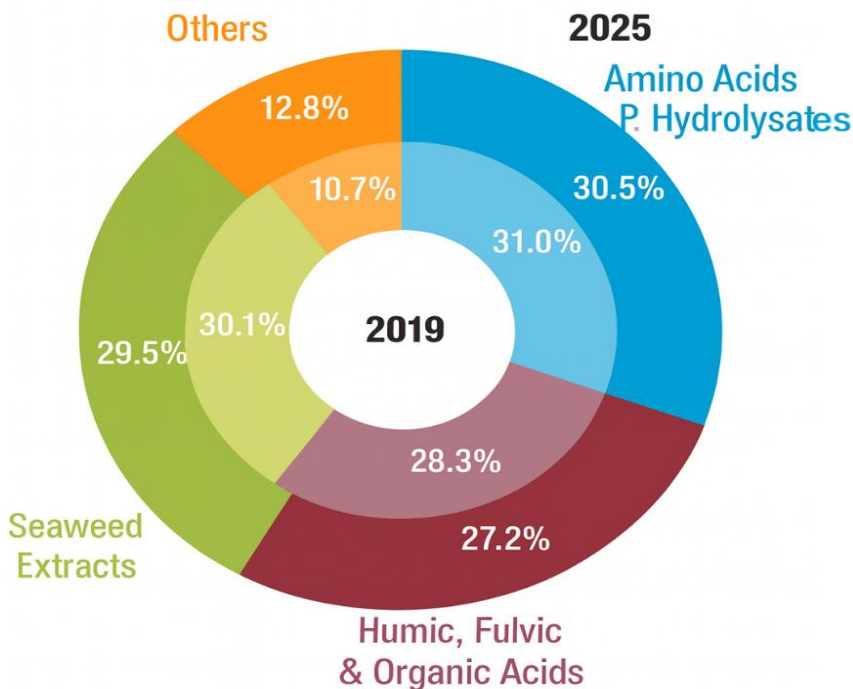
Figure 2: Status of biostimulants in the world market



Source: Dunham Trimer, 2024

Microbial products are the fastest-growing segment in both biological control systems and biostimulants. As shown in Figure 3, conventional biostimulants based on humic acid, fulvic acid, seaweed, and amino acids make up a large portion of this market (Dunham Trimer, 2024).

Figure 3: Market share of conventional biostimulants based on bioaccumulators



Source: Dunham Trimer, 2024

Another meta-analysis published in 2022 by Li et al. evaluated non-microbial biostimulants across 180 studies and reported an average yield advantage of 17.9%. Of course, there is a certain publication bias involved; studies with positive results are much easier to publish than those showing no effect. Therefore, this should be taken into account when interpreting the findings. However, perhaps more interesting is that the highest benefit was obtained from applications in horticultural crops and under suboptimal growing conditions (Li et al., 2022).

In a meta-analysis conducted in 2018 by Schütz et al. focusing on microbial plant biostimulants involving fungi and bacteria, it was found that biostimulants increased phosphorus and nitrogen use efficiency, resulting in an average yield increase of 16% across all crops. Based on 171 field studies, the researchers reported

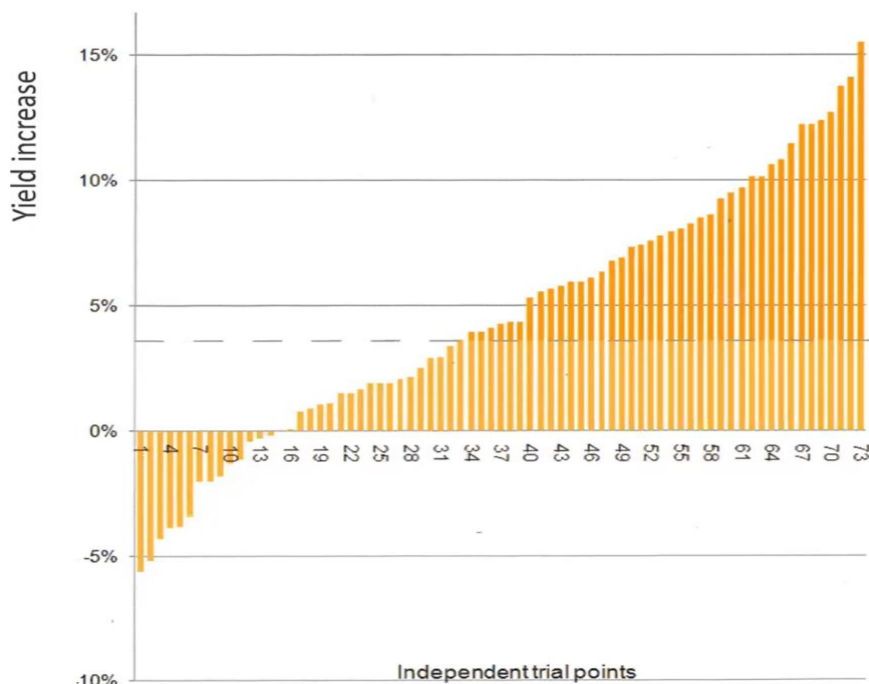
that biofertilizers generally increased crop yield and nutrient use efficiency, with the highest yield increase observed in arid climates. Legumes responded best to biostimulant applications, while low yield performance was observed in root crops. Arbuscular mycorrhizal fungi (AMF) were most effective at low phosphorus levels, while nitrogen fixers were more successful at high phosphorus levels. Combinations of arbuscular mycorrhizal fungi and nitrogen fixers provided higher yield increases than applications alone (Schütz et al., 2018).

Reasons for Regulation Regarding Biostimulants

To ensure the reliability of these products from farmers to end users, specific assurances regarding both their safety and efficacy must be provided. Indeed, in the study published in 1993, which we just mentioned, Kinnersley points out that inconsistent crop performance observed in the field is a significant factor limiting the application of biostimulants in agriculture and horticulture.

To better understand this situation, we can examine the metadata study shared with us by Du Jardin. The study investigated the yield response to a humic/fulvic acid-based biostimulant product tested on winter wheat and applied as a seed coating. A total of 73 trials were evaluated, and the results are given in Figure 4. Both yield increases and decreases were observed in these trials. These results reveal a very wide variability in yield, demonstrating that this is one of the fundamental challenges we all face. Understanding this variability is critically important for both scientists and companies developing products or experts providing technical support to farmers.

Figure 4: Yield response to humic/fulvic acid-based biostimulant product



Source: Du Jardin et al., 2025a

Correct Criteria for Evaluating the Efficacy of Biostimulants

Using the correct criteria to evaluate the effectiveness of biostimulants is crucial. Increased yield in plants is often achieved by improving a number of characteristics such as tolerance to abiotic stress, improved quality traits, availability of limited nutrients, and nutrient use efficiency. However, these characteristics cannot be directly correlated with yield. Therefore, if we are talking about characteristics that can contribute to improving abiotic stress tolerance;

- We need to evaluate criteria such as hormonal balance,
- Root architecture,
- Antioxidants,
- Stomatal arrangements, etc.

These are factors that should be considered separately from yield. While these criteria can certainly be translated into yield, in the absence of abiotic stress factors, yield will not be an accurate criterion for evaluating biostimulants.

To give further examples of appropriate criteria for evaluating the effectiveness of biostimulants, quality characteristics include:

- Phenology
- Total protein content
- Phenolic compound content, etc.

For the availability of limited nutrients;

- Phosphorus solubility
- Assemblage of beneficial microorganisms in the soil and rhizosphere
- Secretion of phytochelates, etc.

For nutrient utilization efficiency;

Many criteria can be mentioned, such as:

- Root development and architecture
- Root activity (cell membrane permeability)
- Redistribution of nutrients within the plant, etc.

Plant biostimulants are defined according to the effects they claim to have, but a clearer definition of the intended effects on plants and products is needed to properly evaluate biostimulants.

Precision Nutrition in Plants with Biostimulants

Plant biostimulants are defined by the effects they claim to have, but to evaluate biostimulants, we need to delve deeper into a

clear definition of their intended effects on plants and crops. Table 1 presents some studies on the targeted effects of biostimulants on agricultural products. Biological stimulants are definitely not a one-size-fits-all solution. You need to know exactly where you want to go. Clearly explaining this to farmers is crucial. However, this is not easy because you need to understand very well which characteristics you want to change and how this can contribute to nutrient use efficiency, for example, nitrogen use efficiency; the individuals and characteristics you will be dealing with can be very complex.

Table 1: Some studies on the effect of agricultural targeted biostimulants

AGRICULTURAL OBJECTIVE	EFFECT OF BIOSTIMULANT	CLAIM
Seedling development, early root development, and nutrient uptake in maize.	Humic acid supports root development and activity (Conselman et al. 2017).	It increases the efficiency of nutrient utilization.
Delayed opening of the soybean capsule.	Seaweed extract delays capsule opening by regulating auxin balance (Langowski et al. 2021).	Ensuring improved quality features.
Increasing drought resistance in tomatoes.	Glycinebetaine-based biostimulant protects photosynthesis against oxidative attacks (Antonucci et al. 2021).	Increased tolerance to abiotic stress.
Higher protein content in wheat grains.	Fermentation byproducts (glutamic and other organic acids) increase nitrogen uptake and utilization (Maignan et al. 2021).	Providing improved quality features.
Etc.	Etc.	Etc.

Source: Du Jardin et al., 2025b

Functional Claims of Biostimulants

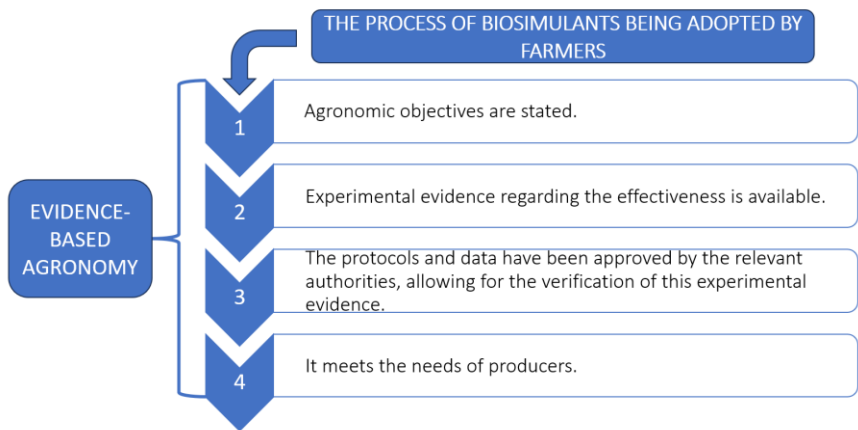
We are moving towards plant-sensitive nutrition, and Patrick Du Jardin has explained this by drawing parallels with human

nutrition and human health. In human nutrition, we have foods that we consume daily, but we also have functional foods. For example, when we consume oatmeal and cereal for breakfast, we are concerned with calories, sugars, and many other things. At the same time, we are also concerned with the physiological activity of oats, such as their capacity to lower blood cholesterol. This physiological goal (lowering blood cholesterol) means that we are talking about functional foods. Here, it has been suggested that biostimulants are actually functional foods for plants and should be distinguished from pesticide-based plant protection products (Du Jardin et al., 2025a).

Adoption of Biostimulants in Evidence-Based Agronomy

The adoption process of biosimulators by farmers relies on evidence-based agronomy. The process is shown in Figure 5. Agronomic goals are defined, experimental evidence of efficacy is available, and protocols and data are approved by competent authorities, allowing for the validation of this experimental evidence. Finally, another important consideration is that the stimulant products meet the needs of the producers.

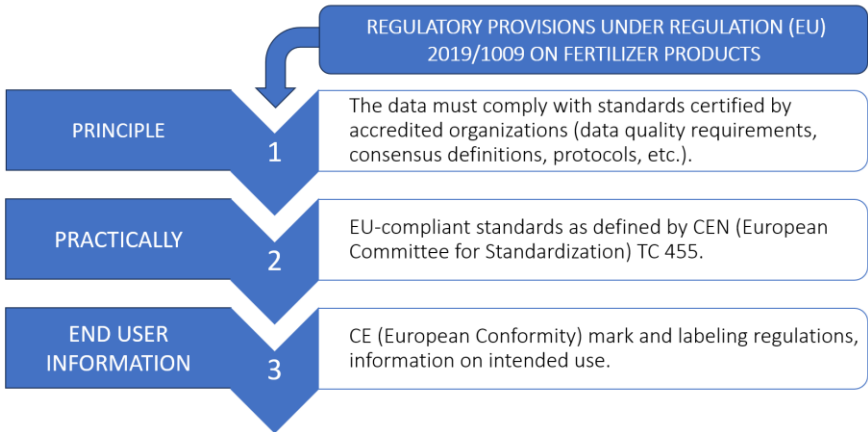
Figure 5: The process of biosimulator adoption by farmers



Source: Du Jardin et al., 2025b

In Europe today, regulatory provisions regarding the conformity of data to standards, certified by approved bodies under the fertilizer products regulation, are given in Figure 6. European Union standards enabling the application of these regulatory provisions are defined. Furthermore, CE marking and labeling provisions introduced by the regulation, according to the intended use of the products, are presented to the end-user. These constitute the key aspects of the regulation and the ways to verify the claims of the products.

Figure 6: Regulatory provisions of the European Fertilizer Products Regulation



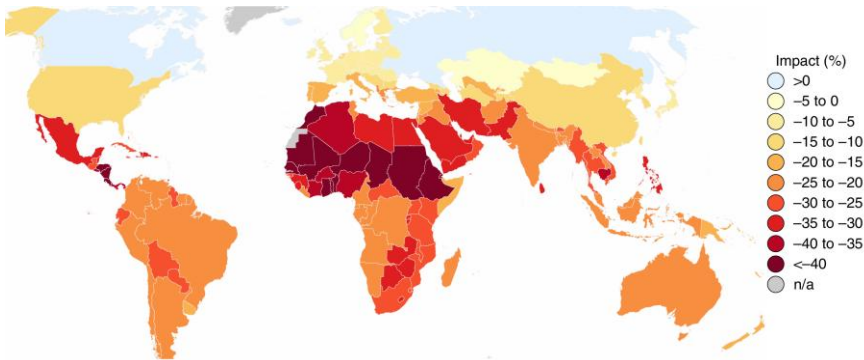
Source: Du Jardin et al., 2025a

Supply and Demand Relationship of Biostimulants

A study on global climate change has shown that over the past 60 years, human-induced climate change has reduced total agricultural production factor productivity globally by approximately 21%, with this negative impact being more

pronounced in warmer regions (Ortiz-Bobea et al. 2021). A visual representation of the study is shown in Figure 7.

Figure 7: Global climate change study



Source: Ortiz-Bobea et al. 2021

It is clear that we need technology, and especially agricultural technology, to cope with this situation. The increase in biological stress creates a supply-demand effect between the scientific community and producers. When the supply from science is considered, the modification of plant development through biological and chemical means, stress memory and chemical adaptation in plants, and the support of plant-microorganism interactions with agricultural inputs are of great importance.

It is possible to alter plant physiology and morphology by applying various substances and microorganisms externally to plants. The use of plant growth regulators and various stimulants activates epigenetic mechanisms by influencing the way genes are expressed without altering the gene sequence. This approach offers highly flexible and versatile tools for controlling gene expression and holds significant potential for developing sustainable and innovative agricultural practices. Plants are known to possess a "memory" mechanism regarding environmental conditions, allowing them to adapt more effectively to stressors through chemical

applications. Furthermore, plants are considered a holobiont along with their associated microorganism communities, and beneficial plant-microorganism interactions can be promoted through appropriate agricultural practices and probiotic/prebiotic agricultural inputs (Brown et al., 2015).

In addition, there is a demand from producers. The expectation is that agriculture will contribute to a sustainable economy by using waste and by-products as fertilizer, reducing nutrient losses from fertilizers, and fostering a societal trend towards more environmentally friendly food production systems. Furthermore, agriculture should incorporate both climate change mitigation and adaptation measures, and provide solutions to the challenging environmental stressors encountered on farms.

Conclusion

Today, the concept of plant health is often viewed negatively, with the absence of diseases resulting from the use of plant protection products considered the primary indicator of health. However, plant health needs to move beyond this narrow definition and be evaluated with a more holistic and positive approach. A positive understanding of plant health refers to the plant's capacity to perform its physiological functions at the highest level in accordance with its genetic potential. In this context, improving plant health is not limited solely to plant protection products; the use of biostimulants can also make significant contributions. Therefore, it is not an approach focused only on disease treatment; it is a multifaceted process that supports growth, development, and stress tolerance. In this process, the use of biostimulants can be considered an effective tool in sustainably improving plant health.

Plant biostimulants are products defined by their claimed agricultural functions, not by their mechanistic modes of action. Their adoption by growers depends on experimental evidence

supported by appropriate regulations regarding efficacy. Biostimulants are expected to contribute to resilient, low-input, high-yield agriculture. One of the key issues here is how to develop precision nutrition in a way that benefits global plant health. It is important for growers to form their own opinions about biostimulant products.

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CHAPTER 6

ELICITORS USED FOR *IN VITRO* DROUGHT STRESS SIMULATION AND PLANT RESPONSES

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Introduction

Plants are exposed to different environmental stress factors throughout their life cycles in natural and agricultural ecosystems. Among these stress factors, drought is one of the most important abiotic stresses limiting plant production and has become an increasingly serious problem worldwide, especially due to the effects of global climate change (Rao et al., 2006; Salehi-Lisar et al., 2016). Rising temperatures, irregular rainfall patterns, and decreasing water resources are increasing the frequency and severity of drought, significantly threatening agricultural production. Since water is an

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indispensable element for the growth and development of plants, water scarcity negatively affects vegetative development, yield, fruit size, and quality characteristics, consequently causing significant economic losses (Şimşek et al., 2018). Drought stress is considered a difficult-to-control environmental stress factor that directly or indirectly affects almost all developmental stages of plants from germination to harvest (İlyas et al., 2021). Therefore, drought, which is becoming increasingly common due to the effects of global climate change, is considered one of the most important abiotic stress factors playing a decisive role in plant growth, development, and agricultural yield (Acemi et al., 2019; Sevindik, 2021).

Increasing the severity of drought stress negatively affects physiological and morphological processes in plants, significantly limiting growth and development, and in advanced stages, can lead to plant death (Ekinçi et al., 2024). Under water scarcity conditions, leaf area, stem elongation, and root development are suppressed, while the plant's water balance is disrupted and water use efficiency is significantly reduced (Farooq et al., 2009; Anjum et al., 2011). At the cellular level, turgor pressure decreases due to water loss, cell volume shrinks, and metabolic activities become difficult to maintain. In addition, drought stress increases the production of reactive oxygen species (ROS) and free radicals, leading to oxidative stress. This situation causes structural and functional damage in essential cellular components such as lipids, proteins, and nucleic acids (Yıldırım et al., 2021). Therefore, in studies aimed at developing drought-resistant plant genotypes, improving root system characteristics that increase water uptake capacity and support the plant's adaptation to stress conditions stands out as an important breeding strategy (Sorkheh et al., 2011).

In drought stress research, *in vitro* tissue culture techniques are widely used to determine stress tolerance and to elucidate physiological and biochemical changes occurring under stressful

conditions. These techniques offer significant advantages in terms of precise control of environmental conditions, simultaneous evaluation of numerous genotypes in limited areas, and reliable experimental replication (Yaman et al., 2020). While a large portion of stress research is conducted in field or greenhouse conditions, these studies have several limitations, such as the inability to control environmental variables, long experimental periods, and high labor and cost requirements. In contrast, *in vitro* culture systems provide controlled experimental conditions, minimizing the impact of external environmental factors and allowing for more reliable and reproducible stress applications (Wijerathna-Yapa and Hiti-Bandaralage, 2023). Furthermore, *in vitro* tissue culture techniques can be applied throughout the year, regardless of the vegetation period of the plants, ensuring uninterrupted research (Ekinci et al., 2024). Thanks to these features, tissue culture methods are considered an important methodological tool for determining tolerance to various abiotic stresses, especially drought, in a short time and for the pre-selection of resistant genotypes (Kara, 2025).

Various osmotic regulators and elicitor agents are used to simulate drought stress under *in vitro* conditions. Among the most commonly preferred compounds for this purpose are mannitol, sorbitol, and polyethylene glycol (PEG). By adding these substances to the nutrient medium in specific concentrations, the water potential in plants is reduced, creating osmotic stress that mimics drought conditions (Meşe and Tangolar, 2019; Bilir and İlhan, 2022). Although natural drought is a complex process resulting from the interaction of numerous environmental factors such as temperature, humidity, light, and soil properties, polyethylene glycol (PEG), a high molecular weight osmotic agent that cannot pass through the cell wall, is widely used in controlled *in vitro* conditions to mechanistically study the physiological and biochemical effects of drought on plants. PEG applications allow for the reliable and

reproducible evaluation of morphological, physiological, and biochemical responses to drought stress by creating controlled water deficiency in plant tissues.

The aim of this review is to examine the main elicitors and osmotic agents used in *in vitro* drought stress induction, to evaluate the physiological, biochemical, and morphological changes these applications cause in plants in light of current literature, and to highlight the importance of *in vitro* approaches for determining drought tolerance.

The Effects of Drought Stress on Plants

Physiological effects (water potential, stomatal closure, photosynthesis)

The first physiological responses to drought conditions include stomatal closure. This occurs due to a decrease in cellular turgor pressure and disrupts the water balance in plant tissues as the plant attempts to reduce water loss through transpiration. This disruption of water balance, over time, impairs enzyme activity and metabolic processes, weakening cellular function and ultimately leading to the appearance of wilting symptoms in the plant (İlhan and Ekbiç, 2023). The decrease in the relative water content (RWC) of leaves is considered one of the significant effects of drought stress on plant physiology (Hemati et al., 2022), and it is known that the root-stem ratio generally increases in plants under water-scarce conditions. The main reason for this is that roots are more tolerant to the growth pressure caused by low water potential than stems and leaves, and under drought stress, root systems create a communication network by transmitting various signaling molecules to shoot regions via the xylem. These signals trigger the plant's stress response mechanisms, regulating adaptation processes at the physiological and developmental levels (Anjum et al., 2011).

Morphological effects (root/shoot development)

Drought stress negatively affects cell division and cell expansion processes, leading to a decline in plant growth. The loss of turgor pressure under water deficiency conditions is one of the main reasons limiting the growth capacity of cells. Furthermore, the inability to transport sufficient water from the xylem tissue to the surrounding cells inhibits cell elongation, thus restricting tissue development. Leaf formation and leaf area also decrease significantly under drought stress. Under normal conditions, leaf expansion depends on maintaining cell turgor and providing sufficient levels of photosynthetic products, but the decrease in turgor pressure and slowing of photosynthetic activity due to water deficiency limit leaf growth and negatively affect the overall development of the plant (Fahad et al., 2017). In a study by Vuksanović et al. (2019), the physiological effects of drought are often observed first in the root system. The decrease in root growth and root number limits the plant's water and mineral uptake capacity, further exacerbating the effects of stress. Indeed, studies conducted under artificial drought conditions created using PEG have shown that increasing PEG concentration leads to significant reductions in shoot length and root number. For example, the application of 50 g/L PEG caused a 51% decrease in shoot length and a 31% decrease in root number. Similar results have been reported in *in vitro* studies on different plant species such as cacti, tomatoes, and strawberries (Kulkarni and Deshpande, 2007; Mengesha et al., 2016; Hussein et al., 2017). These findings reveal that drought negatively affects growth and development in plants by reducing their water potential, leading to morphological changes along with decreased stomatal closure and photosynthesis.

Biochemical effects (ROS, antioxidant system)

Under normal growth and development conditions, ROS are formed in low amounts in cellular organelles such as mitochondria, chloroplasts, and peroxisomes. However, the production of reactive oxygen species increases significantly when plants are exposed to various stress conditions. The increase in reactive oxygen species levels under stress is due to an imbalance between the formation of these molecules and their removal by antioxidant defense mechanisms. The degree of ROS accumulation varies depending on changes in light intensity, temperature, and other environmental factors, the duration and intensity of the applied stress, and the capacity of plant tissues to adapt to the resulting energy imbalance (Miller et al., 2010). However, under abiotic stress conditions such as drought, the production of ROS increases significantly. Excessively accumulated reactive oxygen species within the cell cause oxidative damage to proteins, nucleic acids, and membrane lipids, negatively affecting cellular function. These molecules, which have a high oxidation capacity, promote lipid peroxidation, disrupting the integrity of cell membranes; This can lead to significant damage, ranging from losses in the structural and functional properties of proteins to DNA damage and even advanced cell death (Anjum et al., 2011).

As noted by Impa et al. (2012) and de Carvalho (2008), under drought stress, plants close their stomata to prevent water loss; this restricts the entry of water, leading to a decrease in the rate of photosynthesis and overloading of the photosynthetic electron transport chain. In this process, electron leakage to oxygen leads to the overproduction of ROS species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and the most reactive species, hydroxyl radicals ($HO^\cdot$). *In vitro*, osmotic stress induced by elicitors such as PEG mimics drought by lowering water potential and triggers ROS formation due to cellular dehydration. According to Ahmad et al. (2020), excessive ROS accumulation

leads to lipid peroxidation (LPO), which is usually measured by malondialdehyde (MDA) levels and is considered the most important indicator of cell membrane damage.

Molecular responses (gene expression, stress proteins)

Under conditions of water scarcity, plants develop adaptation mechanisms at the cellular and molecular level by accumulating various osmolytes and protective stress proteins to maintain osmotic balance and reduce the negative effects of stress (Shinozaki and Yamaguchi-Shinozaki, 2007). Furthermore, drought stress plays a significant role in shaping the plant's response to stress by regulating the expression of numerous genes involved in different biological processes (Ding et al., 2013).

The drought stress response in plants is a dynamic process that begins with the sensing of stress in the plasma membrane and triggers complex signal transduction pathways, leading to radical changes in gene expression (Singh et al., 2023). These molecular mechanisms enable the plant to adapt to water scarcity, repair cellular damage, or activate protective systems. When drought signals are detected, plants generally develop molecular responses via two main pathways: abscisic acid (ABA)-dependent and ABA-independent. *In vitro* studies, particularly on banana plants (*Musa L.*), have shown that the calcium-dependent protein kinase (CDPK) gene family plays a critical role in signal transduction, and that the expression levels of MaCDPK1, MaCDPK9, MaCDPK21, and MaCDPK40 genes change significantly in a tissue-specific manner under drought stress (Zekai et al., 2022). Molecular analyses performed on maize plants revealed that the expression levels of transcription factors such as ZmbZIP33, ZmNAC052, and ZmMYB-R1 increase under drought stress, and that these genes enhance the plant's ability to cope with stress via ABA signaling pathways (Singh et al., 2023). These factors, which play a key role in the regulation

of gene expression, activate plant protection mechanisms, ensuring osmotic balance and cellular integrity. In drought-sensitive species, the expression of enzymes such as chlorophyllase, which degrade chlorophyll under stress, can increase, leading to damage to the photosynthetic apparatus (Rokosa et al., 2025).

To provide protection at the cellular level, plants synthesize specific stress proteins such as Heat Shock Proteins (HSP) and Late Embryogenesis Bol Proteins (LEA). Proteomic analyses show that these proteins stabilize cellular structures during dehydration and prevent protein misfolding or aggregation (Singh et al., 2023). In addition, the activity of antioxidant enzymes such as peroxidase (POD) for ROS detoxification is increased at the molecular level (Singh et al., 2023). The use of osmotic agents such as PEG or sorbitol as elicitors to simulate drought stress *in vitro* allows for rapid screening of these molecular responses in a controlled environment (Albiski et al., 2012). Studies on guava plants have shown that using 8% PEG allows for the identification of specific DNA bands between drought-tolerant and drought-sensitive lines via molecular markers such as SRAP and ISSR, proving these markers to be effective tools in molecular breeding programs (Abouzaid et al., 2016). In summary, molecular responses under drought stress are a complex network characterized by signal sensing, transcriptional rearrangement, and the synthesis of protective stress proteins (Abouzaid et al., 2016; Zekai et al., 2022).

Simulation of Drought Stress Under *In Vitro* Conditions

Tissue culture methods are widely used in the development of plants tolerant to abiotic stress conditions. The main reason for this is that environmental factors can be controlled more precisely in *in vitro* systems than *in vivo* conditions, and studies can be conducted on a large number of materials in small areas (Yaman et al., 2020). The main aim in creating drought stress *in vitro* conditions is to

mimic the cellular and physiological conditions that plants encounter in their natural environment under water scarcity in a laboratory setting. When the water potential in cells decreases due to drought, plants accumulate various dissolved substances and osmoprotective compounds to maintain osmotic balance. Therefore, osmotic agents such as sucrose, sorbitol, mannitol, and PEG are prevalent used to create drought-like conditions by reducing the water potential of the culture medium. In addition, these compounds contribute to the preservation of cell membrane integrity and the stabilization of protein structures under water deficiency conditions, thus supporting the stress tolerance of plants (Wijerathna-Yapa & Hiti-Bandaralage, 2023).

Advantages of *in vitro* techniques

In vitro propagation is widely used for the rapid propagation of many plant species (Hazarika, 2006) and is of great importance because it allows production regardless of the vegetation period and ensures the obtainment of clean plant material free from diseases and pests (Ekinici et al., 2025).

The use of tissue culture techniques under *in vitro* conditions provides significant advantages in studies aimed at evaluating the stress tolerance of plants, revealing the physiological and biochemical changes caused by stress, and increasing tolerance levels. Although a large part of stress research is carried out under field or greenhouse conditions, such studies have various limitations such as high cost, the need for large areas, and the inability to control environmental variables. In contrast, *in vitro* systems allow for the precise control of environmental factors, enable working with a large number of samples in a smaller area, and allow research to be carried out more economically (Wijerathna-Yapa and Hiti-Bandaralage, 2023). *In vitro* tissue culture techniques are preferred because they provide continuity of study under controlled conditions and are

independent of the vegetation period of the plants (Ekinici et al., 2024).

Elicitors that Induce Drought Stress

In vitro approaches offer a more controlled and practical research environment compared to field trials in evaluating drought tolerance in plants. The main purpose of *in vitro* drought simulation is to recreate the cellular and physiological processes that occur in plants experiencing water scarcity under natural conditions in a laboratory environment. Under water restriction, plants accumulate various dissolved substances and osmoprotective compounds to maintain intracellular osmotic balance. Therefore, substances such as sucrose, sorbitol, mannitol, and PEG are commonly used to create drought-like conditions by reducing the water potential of the culture medium. In addition, these compounds contribute to the preservation of cell membrane integrity and the stability of protein structures during the dehydration process, thus playing a role in reducing the negative effects of stress (Wijerathna-Yapa and Hiti-Bandaralage, 2023).

Polyethylene Glycol (PEG)

Drought, one of the leading environmental challenges, when combined with stress factors such as high temperature or salinity, leads to significant water loss, or dehydration, in plant cells (Bartels and Sunkar, 2005; Verslues et al., 2006). This process is mostly triggered by osmotic signals at the cellular level and forms the basis of the plant's defense mechanisms against drought (Martignago et al., 2020).

Osmotic stress, which develops as a result of the disruption of the osmotic gradient in intracellular and extracellular environments, necessitates extremely rapid cellular responses for the plant to maintain cellular balance and adapt to harsh environmental conditions (Haswell and Verslues, 2015; Yu et al., 2024).

Fully studying the natural responses of plants to drought is only possible through field trials. In these studies conducted in soil environments, the aim is to limit or completely cut off the water inflow from precipitation. However, climatic fluctuations and unpredictable rainfall amounts in natural ecosystems significantly complicate the reproducibility of these experiments (Tuberosa, 2012).

To overcome this instability, achieve high reproducibility in experiments, and efficiently utilize modern phenotyping technologies, artificially dried soil systems in controlled areas such as laboratories, greenhouses, or climate chambers are considered more advantageous (Negin and Moshelion, 2016).

These studies, conducted using pots and similar containers in greenhouses, grow rooms, or laboratory benches, technically fall into the category of "artificially controlled drought" when compared to natural drought. This technical definition is rarely encountered in the literature, as it is usually clearly stated whether the research was conducted in field or off-field conditions.

The situation takes on a different dimension when it is desired to restrict the amount of water that the plant can absorb using chemical methods. The most frequently used chemical agent for this purpose is polyethylene glycol, which usually has a high molecular weight (6000 or 8000). PEG, a neutral polymer, forms strong hydrogen bonds with water molecules, reducing the potential for available free water in the environment and thus creating an artificial water scarcity scenario (Hanász et al., 2022; Mozafari et al., 2018; Vuksanović et al., 2023).

The key features that distinguish PEG from other low molecular weight osmotic substances and make it advantageous are:

- **Inability to Cross the Apoplast Barrier:** Due to its large molecular structure, it cannot penetrate the cell wall (enter the apoplast).

- **Realistic Dehydration Simulation:** Since it does not penetrate the cell, it creates a natural dehydration effect by drawing water from outside the cell, just like in drying soil (Van den Berg and Zeng, 2006).

- **High Efficiency and Speed:** It offers a practical, economical, and fast model, allowing for the rapid screening and evaluation of the drought resistance of numerous plant genotypes (Parlar et al., 2025).

Mannitol

Mannitol is a six-carbon sugar alcohol widely used in plant physiology research, particularly to simulate drought and osmotic stress under *in vitro* conditions (Lipavská and Vreugdenhil, 1996; Liu et al., 2015). It is considered an ideal material for simulating soil drought primarily because it is a high molecular weight osmotic regulator and has a low toxicity structure that is presumed to be inaccessible to cell walls (Liu et al., 2015). Thanks to these properties, researchers can study the morphological and biochemical responses under stress of strategically important plants such as maize, sugarcane, rice, and jojoba by controllably reducing the water potential of the culture medium (Roussos, 2013; Chutipaijit, 2016; Snyman et al., 2016).

For many years, mannitol was assumed to be an inert substance that could not be metabolized by plants and therefore only affected the osmotic potential of the environment (Lipavská and Vreugdenhil, 1996). However, current research shows that this assumption is not always valid; studies by Lipavská and Vreugdenhil (1996) on wheat embryos, rapeseed seedlings, and potato stem segments have shown that mannitol is readily taken up by plant

tissues and rapidly transported from roots to shoots. The accumulation of mannitol in plant tissues can reach 20% to 30% of dry weight, indicating that this compound should be considered not only as an exogenous osmotic agent but also as an active component capable of influencing intracellular balance (Lipavská and Vreugdenhil, 1996). These findings suggest that *in vitro* studies, mannitol should be evaluated not only as an exogenous osmotic agent but also as a component incorporated into the plant's internal structure and capable of influencing intracellular balance. Mannitol-simulated drought stress leads to significant changes in plant morphology. Liu et al. (2015) showed that increasing mannitol concentrations severely reduced germination rate, germination potential, and seedling growth rate in maize varieties. Similarly, shortening of shoot number and height was observed in jojoba explants, while rooting success decreased as the severity of stress increased, as determined by Roussos (2013). Nevertheless, plants can develop adaptive mechanisms by increasing the root-shoot ratio (Liu et al., 2015). In particular, the root regrowth potential test applied by Snyman et al. (2016) in sugarcane is an effective screening tool that provides results in as little as 3 weeks for determining the drought tolerance of varieties. Plants also activate defense systems at the biochemical level against mannitol stress. The accumulation of compatible solutes such as proline to balance intracellular water loss is one of the key osmotic regulation strategies highlighted by Roussos (2013). While drought stress causes ROS overproduction and oxidative damage in cells, Chutipaijit (2016) found that tolerant rice genotypes combat this damage more effectively by increasing the antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Furthermore, as seen in Roussos' (2013) study, the increase in glucose levels and decrease in sucrose levels are important indicators that the plant is reorganizing its energy sources under stress conditions. Consequently, mannitol is an indispensable tool for

the rapid selection of drought-resistant genotypes in plant breeding studies (Snyman et al., 2016). Although the responses of different plant species to this stress show a high correlation with field performance, the fact that mannitol can be absorbed by the plant, as noted by Lipavská and Vreugdenhil (1996) and Roussos (2013), is critical for the correct interpretation of the data.

Sorbitol

Sorbitol is a sugar alcohol derived from glucose and highly soluble in water. While its sweetness level is lower compared to sucrose, it is naturally synthesized in some plant species. Due to its capacity to regulate osmotic balance, sorbitol is one of the osmotic agents frequently preferred for creating drought-like conditions in *in vitro* studies (Mehmandar et al., 2023).

In plant physiology studies, it is considered one of the fundamental osmotic agents (exhibiting elicitor-like effects) widely used, especially for simulating drought stress in *in vitro* conditions. Sorbitol creates osmotic stress in plant cells by lowering the water potential of the culture medium, thus allowing the study of physiological responses similar to natural environmental drought conditions in a laboratory setting (Gopal and Iwama, 2007; Razavizadeh and Adabavazeh, 2017; Akın, 2022).

Sorbitol has certain advantages compared to substances like PEG, with which it is frequently compared in drought simulation. Due to its high viscosity, PEG can restrict oxygen movement in the environment and lead to toxic effects due to oxygen deficiency in the roots. In contrast, sorbitol offers a more flexible structure that is easily washed away from the roots, is less viscous, and can be partially absorbed by the plant at low concentrations (Sajid and Aftab, 2022). However, the effect of sorbitol is dose-dependent; low concentrations (e.g., 10 mM in maize) can act as an osmoprotectant

against drought and support growth, while high concentrations create a suppressive stress factor on growth (Li et al., 2023).

Literature Review and Summary of Studies

Drought stress is one of the most common abiotic stress factors, having significant limiting effects on growth, development, and yield in plants. Water scarcity causes many physiological and biochemical changes. Therefore, numerous studies have been conducted in different species to understand the effects of drought stress on plants. Some of these studies are given below.

Table 1 Literature Review

Author (Year)	Plant Material / Stress Factor	Applied Dose	Parameters Evaluated	Results
Akçay and Koç (2026)	<i>Phaseolus vulgaris</i> L. / PEG + SA	PEG6000 (−0.351 and −0.75 MPa); SA (1 mM)	SL, RWC, Chl <i>a</i> , MDA, H ₂ O ₂	Drought stress negatively affected growth and physiological traits, whereas SA application alleviated drought stress by reducing oxidative damage.
Kara (2025)	Radish ('Kırmızı İnci', 'Chery Belle') / PEG	0%, 2%, 4%, 6%	G, SL, FW, DW	Increasing PEG concentration reduced growth parameters. The 'Kırmızı İnci' cultivar exhibited higher drought tolerance than 'Chery Belle'.
Parlar et al. (2025)	Cornerstone and GF-677 rootstocks / PEG 8000	Different PEG 8000 concentrations	ShG, Chl, RWC, Pro, FW, DW	PEG treatment reduced growth. The Cornerstone rootstock showed higher drought tolerance than GF-677 through greater proline accumulation, lower chlorophyll loss, and better dry matter preservation.
Mehmandar et al. (2023)	<i>Cucumis melo</i> L. / Sorbitol and PEG	Sorbitol (0.1–0.4 M); PEG (0.009–0.015 M)	G, PP, Pro, MDA, Prot, AEA	Sorbitol and PEG reduced growth and photosynthetic pigments while increasing proline and MDA levels. Sorbitol was found to simulate drought stress more effectively than PEG.
Mohsen et al. (2020)	1103P, 140 RU, Ramsey and Dog Ridge / PEG	0%, 1.5%, 3%, 4.5%, and 6%	G, Chl, Pro, SR, LA, DSI	PEG treatment reduced growth and chlorophyll content while increasing proline accumulation, leaf abscission, and drought stress index. Ramsey rootstock exhibited the highest drought tolerance.
Acemi et al. (2019)	<i>Amsonia orientalis</i> / PEG-6000	Osmotic potentials of −0.15,	SN, ShL, H ₂ O ₂ , MDA,	Drought stress suppressed shoot growth, increased H ₂ O ₂ and MDA levels, and reduced

		-0.49, -1.03, and -1.76 MPa	SOD, POD, CAT	antioxidant enzyme activities. BAP treatment supported non-enzymatic defense mechanisms.
Meşe and Tangolar (2019)	Kober 5 BB, 110 R, and 1103 P grapevine rootstocks / PEG	0%, 2.5%, 5.0%, 7.5%, and 10% PEG	DR, PH, NN, RL, RN, FW, DW, Chl, NC	Increasing PEG concentrations negatively affected growth parameters and chlorophyll content. PEG concentrations of 2.5% and 5% were found suitable for early selection of drought tolerance.
Şimşek et al. (2018)	Troyer citrange and C-35 citrange (citrus rootstocks) / <i>In vitro</i> PEG 8000 (polyethylene glycol)	0%, 1%, 2%, 4%, and 6% PEG 8000	PP, G, V	Plants maintained viability and propagation under increasing PEG doses, although growth and propagation performance decreased markedly.
Jolayemi and Opabode (2018)	<i>Manihot esculenta</i> (cassava), 12 cultivars / Mannitol	0 g/L and 20 g/L mannitol (MS medium)	SN, SDW, LN, RL, RDW, STI	Mannitol increased shoot number but reduced shoot dry weight and leaf number. Root development varied depending on genotype. Based on the stress tolerance index, TMS 84/00353, MM 96/1751, MM 98/3437, and TMS 94/0026 were identified as drought-tolerant cultivars.
Jolayemi et al. (2018)	<i>Manihot esculenta</i> Crantz (cassava) / Mannitol	Mannitol (0, 5, 10, 15, 20, 25, and 30 g/L)	EV, LN, CLN, RP, RN, RL, ShL	Increasing mannitol levels adversely affected shoot growth and explant viability, and differences in drought tolerance were observed among genotypes. CH140 was identified as the most tolerant cultivar, and 20 g/L mannitol was found to be an appropriate concentration for inducing <i>in vitro</i> drought stress.
Karimi et al. (2012)	Iranian almond cultivars ('Mamaei', 'Sepid', and 'B-124'), two foreign almond cultivars ('Supernova' and 'Ferragnès'), and GF677 rootstock / PEG	Three PEG levels in MS medium (0%, 3.5%, and 7%) for 40 days	GP, RWC, MSI, Pro, LSN	Under PEG-induced drought stress, Supernova and GF677 were tolerant, B-4 and Sepid were moderately sensitive, whereas Mamaei and Ferragnès were sensitive. Leaf abscission, apical meristem necrosis, and poor lateral shoot formation were pronounced in sensitive genotypes, while growth index decreased in all genotypes.
Sotiropoulos et al. (2006)	Apple rootstocks (<i>Malus</i> spp.; M9 and MM106) / Sorbitol	Sucrose: 44, 88, 176 mM; Sorbitol: 82, 164, 329 mM	SMR, ShL, FW, MC, POD, CAT	The M9 rootstock exhibited the highest shoot number and shoot length at 164 mM sorbitol, whereas MM106 showed the highest fresh weight and shoot growth at 329 mM sorbitol. Sorbitol treatments increased

				peroxidase and catalase activities in both rootstocks, with genotype-dependent responses.
Gangopadhyay et al. (1997)	<i>Brassica juncea</i> (L.) Czern. var. RW-85-59 callus culture / Mannitol	Mannitol: 165 and 329 mM; NaCl: 43 and 171 mM	CG, Pro, API	Adapted calli maintained growth, exhibited increased proline accumulation and acid phosphatase isoenzyme activity, and showed greater tolerance to osmotic shock.

Note: AEA, antioxidant enzyme activities; API, acid phosphatase isoenzymes; BAP, 6-benzylaminopurine; CAT, catalase; CG, callus growth; Chl, chlorophyll; Chl a, chlorophyll a; CLN, chlorotic leaf number; DR, damage rate; DSI, drought stress index; DW, dry weight; EV, explant viability; FW, fresh weight; G, germination/growth; GP, growth parameters; H₂O₂, hydrogen peroxide; LA, leaf abscission; LN, leaf number; LSN, lateral shoot number; MC, mineral content; MDA, malondialdehyde; MSI, membrane stability index; NC, nutrient content; NN, node number; PH, plant height; POD, peroxidase; PP, photosynthetic pigments/post-germination propagation performance; Pro, proline; Prot, protein content; RDW, root dry weight; RL, root length; RN, root number; RP, rooting percentage; RWC, relative water content; SDW, shoot dry weight; SG, seedling growth; ShG, shoot growth; ShL, shoot length; SL, seedling length; SMR, shoot multiplication rate; SN, shoot number; SOD, superoxide dismutase; SR, survival rate; STI, stress tolerance index; V, viability.

Table 1 shows that polyethylene glycol (PEG) and mannitol are most commonly used in inducing *in vitro* drought stress, while sorbitol has been considered as an alternative osmotic agent in some studies (Gangopadhyay et al., 1997; Sotiropoulos et al., 2006; Mohsen et al., 2020; Mehmandar et al., 2023). The vast majority of studies reported that increased osmotic stress levels lead to decreased germination, shoot and root development, biomass production, and chlorophyll content (Şimşek et al., 2018; Meşe and Tangolar, 2019; Mohsen et al., 2020; Kara, 2025; Parlar et al., 2025). In contrast, it has been determined that under drought stress, stress markers such as proline, H₂O₂, and MDA increase, activating various defense mechanisms of plants against oxidative damage (Acemi et al., 2019; Mehmandar et al., 2023; Parlar et al., 2025). Furthermore, it has been reported that the application of growth regulators such as

salicylic acid (SA) and 6-benzylaminopurine (BAP) can increase the resistance of plants to drought stress by reducing oxidative damage (Acemi et al., 2019; Akçay and Koç, 2026).

Studies conducted under *in vitro* conditions offer significant advantages in determining the physiological and biochemical effects of drought stress, as well as in the early selection of drought-tolerant genotypes. Research conducted on different plant species has reported that the Red Pearl radish variety, Cornerstone and Ramsey rootstocks, CH140 cassava variety, and Supernova and GF677 almond genotypes exhibit higher tolerance to drought stress (Karimi et al., 2012; Jolayemi et al., 2018; Mohsen et al., 2020; Kara, 2025; Parlar et al., 2025). However, it has been shown that sorbitol simulates drought stress more successfully than PEG in some species, and that appropriate concentrations of PEG or mannitol can be used as reliable stress inducers for early determination of drought tolerance (Meşe and Tangolar, 2019; Jolayemi et al., 2018; Mehmandar et al., 2023). Overall, *in vitro* culture systems stand out as an effective research approach for determining drought-resistant plant material and elucidating stress tolerance mechanisms due to their ability to provide controlled experimental conditions, precisely adjust stress intensity, and compare numerous genotypes in a short time.

Conclusion and Future Perspectives

Drought stress, increasingly prevalent due to the effects of global climate change, is one of the most significant abiotic stress factors limiting plant production, vegetative growth, and crop quality. Drought research conducted under field conditions has several limitations due to the inability to control environmental variables, seasonal variations, and high costs. In contrast, *in vitro* tissue culture techniques offer significant methodological advantages in drought research because they allow for controlled

experimental conditions, high reproducibility, and the evaluation of a large number of genotypes in a short time. The most commonly used osmotic agents in creating drought stress in these systems are PEG, mannitol, and sorbitol. PEG, due to its high molecular weight, reduces the water potential of the environment without penetrating plant cells, thus simulating natural drought conditions more realistically, while mannitol and sorbitol can elicit different physiological responses depending on the plant species. Studies examined show that these elicitors suppress growth and development in plants, while activating proline accumulation, the antioxidant defense system, and other stress response mechanisms, making them effective tools in evaluating drought tolerance. Future research should prioritize multiple stress approaches that consider drought in conjunction with temperature, salinity, and other abiotic stress factors to better reflect natural environmental conditions. Furthermore, detailed investigation of the morphological, physiological, biochemical, and molecular responses of plants to osmotic elicitors using high-throughput omics technologies such as transcriptomics, proteomics, and metabolomics, along with AI-assisted phenotyping and digital imaging systems, will contribute to a more comprehensive understanding of drought tolerance mechanisms. In addition, validating the resistance mechanisms and molecular markers identified under *in vitro* conditions through field trials will accelerate the development of new climate change-resistant plant genotypes, significantly contributing to sustainable agricultural production.

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CHAPTER 7

GEOCHEMICAL ASSESSMENT OF HEAVY METAL CONTAMINATION IN SEMI-ARID SOILS: POLLUTION INDICES, BACKGROUND SELECTION, AND SOURCE APPORTIONMENT

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Introduction

Soil is simultaneously a sink, a buffer, and a secondary source for trace elements. Unlike many organic contaminants, potentially toxic elements (PTEs) are not biodegradable; once deposited, they are redistributed among soil phases but not destroyed, so the contamination signal persists across decades. In semi-arid regions this persistence is amplified by characteristic pedoenviromental conditions: limited leaching under low precipitation, alkaline and calcareous matrices that immobilise several cationic metals, high evaporative concentration of soluble salts, and sparse vegetation

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cover that exposes surface horizons to wind erosion and resuspension. Where such soils surround heavy industry, particularly petroleum refining and associated transport corridors, the question is rarely whether elevated metal concentrations exist but whether those concentrations reflect the natural geochemistry of the parent material or a measurable human contribution.

Answering that question rigorously requires more than reporting concentrations against a regulatory guideline. A measured lead value of 90 mg kg^{-1} is neither inherently safe nor inherently alarming; its meaning depends on the local lithology, the analytical fraction measured, the reference value against which it is compared, and the spatial and statistical structure of the wider dataset. The discipline has therefore converged on a family of normalised indices that translate raw concentrations into interpretable measures of enrichment, contamination, and ecological risk. Each index encodes assumptions, and the credibility of a soil quality assessment rests on making those assumptions explicit and defensible.

The global significance of the problem is well established. Soil contamination by trace elements affects agricultural productivity, threatens groundwater through leaching, and exposes populations through ingestion of soil and dust, dermal contact, inhalation of resuspended particles, and consumption of crops grown on contaminated land. Petroleum refining is a recognised source of a characteristic suite of elements: lead and other metals historically associated with fuel additives and combustion, cadmium and vanadium from crude oil and catalysts, and a range of metals carried in particulate emissions, spent catalysts, and process residues. Refineries are also point sources of total petroleum hydrocarbons (TPH), which frequently covary with the metal signal and provide an independent marker of process-related contamination. The co-occurrence of an enriched metal suite and elevated hydrocarbons is therefore a strong indicator of a refining footprint.

Semi-arid settings modify how this footprint is expressed and detected. Low organic matter limits the soil's capacity to complex and retain metals in stable forms, while high pH and abundant carbonate favour precipitation and surface sorption of cationic metals, reducing their mobility but concentrating them in surface horizons. Salinity, expressed as elevated electrical conductivity, can compete for sorption sites and influence speciation. Sparse vegetation and episodic high-energy wind events promote the redistribution of fine, metal-enriched particles, so that the spatial pattern of contamination reflects both the location of sources and the prevailing transport pathways. These conditions make surface soils in semi-arid industrial regions an especially informative and especially fragile recording medium.

This chapter is organised as a workflow. Section 2 establishes the conceptual distinction between geogenic and anthropogenic signals. Section 3 addresses the sampling and analytical decisions that constrain interpretation. Section 4 treats background selection, the choice that most strongly governs every index value. Sections 5 and 6 develop single-element and integrated indices respectively. Section 7 describes multivariate source apportionment and Section 8 the geostatistical mapping of spatial structure. Section 9 presents a worked semi-arid example, and Section 10 discusses limitations and good practice before the chapter links forward to human health risk assessment.

2. Geogenic and Anthropogenic Signals

Every element measured in a soil sample has two notional contributions: a lithogenic fraction inherited from the weathering of parent rock, and an anthropogenic fraction added by human activity. The lithogenic fraction is governed by mineralogy. Elements such as nickel, chromium, cobalt, and vanadium are concentrated in mafic and ultramafic minerals and in clays; where these dominate the

parent material, soils carry naturally elevated concentrations of those elements without any pollution. By contrast, elements such as lead, cadmium, zinc, and certain metalloids are comparatively scarce in average crust and are strongly associated with specific industrial processes, so their enrichment above background is a more reliable indicator of human input.

Source assignment is also informed by the geochemical behaviour of each element. Anthropogenic metals deposited at the surface tend to reside in more labile, non-residual phases, whereas lithogenic metals are locked in resistant mineral lattices. Although a full sequential extraction is required to demonstrate phase partitioning directly, the expectation that recently deposited anthropogenic metals are comparatively mobile, while inherited metals are residual, underlies the interpretation of both index values and correlation structure. In alkaline semi-arid soils, however, even anthropogenic cationic metals may be partly immobilised by carbonate precipitation, which suppresses leaching but preserves the surface enrichment that indices detect.

A further consideration is that no element is purely diagnostic in all settings. Lead is an excellent anthropogenic tracer in most contexts but can be lithogenically elevated over galena-bearing or mineralised terrain; nickel and chromium are usually lithogenic but become anthropogenic near metallurgical or tannery sources. The correct categorisation is therefore always site-specific and must be argued from the local lithology and the convergence of multiple indicators, never assumed from the element's identity alone.

3. Sampling and Analytical Considerations

Index values inherit every limitation of the data from which they are computed; no statistical treatment can recover information that the sampling design failed to capture. Four decisions are decisive in semi-arid settings.

3.1 Sampling depth and grain size

Surface horizons (0–20 cm) integrate atmospheric deposition and surface runoff and are the appropriate medium for detecting recent anthropogenic loading. Because trace metals concentrate preferentially in fine particles owing to their high specific surface area, the analytical fraction must be standardised; the fine fraction (commonly < 2 mm for total digestions, or < 63 μm where comparability with sediment studies is sought) should be specified and held constant across all samples. Mixing fractions across sites is a frequent and avoidable source of spurious spatial pattern.

3.2 Digestion and the meaning of total versus available

A strong-acid total or pseudo-total digestion (for example aqua regia, or HF-based total dissolution) quantifies the element inventory and is the basis for geoaccumulation and enrichment indices, which are defined against crustal or lithological references. Single- or sequential-extraction schemes instead estimate the mobile or bioaccessible pool relevant to plant uptake and exposure. The two answer different questions; an index computed on a pseudo-total digestion must not be interpreted as a statement about bioavailability.

3.3 Quality assurance

Certified reference materials, procedural blanks, and replicate analyses are not optional embellishments but the only evidence that measured concentrations are accurate. Recoveries for certified materials should be reported, and detection limits stated, because index values near the background are sensitive to analytical error. In alkaline, carbonate-rich semi-arid soils, matrix effects on instrumental determination (for example calcium interference in some spectrometric methods) deserve explicit attention.

3.4 Spatial design and sample support

Because contamination in industrial landscapes is spatially structured, the configuration of sampling points determines whether that structure can be resolved. A design that combines a coverage component, ensuring that the whole study area is represented, with a clustered component near suspected sources, capturing short-range variability, is generally more informative than either a regular grid or purely judgemental sampling alone. The number and spacing of points set the limit on the spatial detail that geostatistical interpolation (Section 8) can recover; too few points, or points clustered only near sources, leave the periphery poorly constrained and inflate interpolation uncertainty. Coordinates must be recorded in a defined projected coordinate system so that distances, and therefore spatial models, are metric and reproducible.

3.5 Data treatment before indexing

Several preprocessing decisions affect every subsequent calculation and statistic. Values below the analytical detection limit (non-detects) cannot simply be discarded or set to zero without bias; common conventions substitute a fraction of the detection limit, but the chosen rule and its effect on elements with many non-detects should be reported. Trace-element distributions in soils are typically right-skewed, so summary statistics and multivariate methods that assume normality benefit from a logarithmic transformation, and the use of the geometric rather than arithmetic mean is often more representative of central tendency. Outliers must be examined rather than reflexively removed, since in a contamination study the highest values are frequently the most informative observations, marking the location of a source. Finally, soil composition is closed data that sum to a constant, which can induce spurious negative correlations; this closure effect should be borne in mind when interpreting the correlation and factor structure of Section 7.

4. Selecting a Geochemical Background

The reference concentration against which a soil value is normalised is the single most influential parameter in the entire assessment. Because the geoaccumulation index, enrichment factor, and contamination factor are all ratios to a background, an inappropriate reference can shift an element by one or more contamination classes and invert a source interpretation. Three families of reference exist.

4.1 Global crustal averages

Compilations of average upper continental crust composition provide a convenient, universally available baseline. The classic tabulations of Turekian and Wedepohl (1961) and the crustal estimates of Wedepohl (1995), together with the upper crustal values of Taylor and McLennan (1985) and the later synthesis of Rudnick and Gao (2003), are the most widely cited. Their advantage is comparability across studies; their weakness is that average crust may differ markedly from the actual parent material of a specific site. Using a global average for a soil developed on carbonate or evaporite-influenced parent material, common in semi-arid basins, can manufacture apparent depletion or enrichment that is purely an artefact of the reference choice.

4.2 Regional and local baselines

A regionally determined baseline, derived from uncontaminated soils of the same lithology and climate, is almost always preferable when available. Regional baseline studies of the relevant geological province should be sought first, and a global average used only as a transparent fallback. Where a study reports indices against both a regional baseline and a crustal average, the divergence between the two is itself informative about the suitability of the global reference.

4.3 Statistically derived thresholds

Where no external baseline is reliable, the background can be estimated from the dataset itself. The least-contaminated samples, identified through probability plots, the iterative 2-sigma technique, or the median of the lower mode in a bimodal distribution, approximate a local natural concentration. A normalisation element that is essentially conservative and lithogenic, commonly aluminium, iron, or titanium, can also anchor the enrichment factor to grain-size and mineralogical variation. Whichever route is chosen, the background and its provenance must be stated explicitly so that index values can be recomputed by any reader.

4.4 Indices versus regulatory guidelines

A recurring error is to use a regulatory soil quality guideline as the background in a geoaccumulation or enrichment calculation. Guideline values, such as the Canadian soil quality guidelines or comparable national standards, are risk-based or effects-based thresholds derived to protect a particular receptor or land use; they are not estimates of natural concentration. Substituting a guideline value for a geochemical background conflates two distinct questions, how enriched a soil is relative to nature, and whether it exceeds an acceptable threshold, and typically produces index values that are neither interpretable nor comparable. Guidelines have a legitimate and separate role: once contamination has been quantified against an appropriate geochemical background, the measured concentrations, not the indices, should be compared with the relevant guideline to judge regulatory exceedance. Keeping the geochemical and regulatory comparisons distinct is essential to a defensible assessment.

5. Single-Element Indices

5.1 Contamination factor

The contamination factor (CF) is the simplest normalisation, the ratio of the measured concentration of element *i* to its background concentration:

$$CF_i = C(\text{sample}, i) / C(\text{background}, i)$$

Håkanson (1980) classified CF into four bands: $CF < 1$ indicates low contamination; $1 \leq CF < 3$ moderate; $3 \leq CF < 6$ considerable; and $CF \geq 6$ very high contamination. The contamination factor is the building block of several integrated indices discussed in Section 6.

5.2 Geoaccumulation index

The geoaccumulation index, introduced by Müller (1969), remains the most widely used single-element measure. It compares the measured concentration with 1.5 times the background, the factor of 1.5 accommodating natural lithological variability so that minor fluctuations are not read as pollution:

$$I_{geo} = \log_2 [C(\text{sample}) / (1.5 \times C(\text{background}))]$$

Because of the base-2 logarithm, each unit increase in I_{geo} corresponds to roughly a doubling of concentration relative to background. Müller's seven-class scheme (Table 1) is the standard interpretive framework.

Table 1. Müller (1969) geoaccumulation index classification.

Igeo value	Class	Soil quality
$I_{geo} \leq 0$	0	Practically uncontaminated
$0 < I_{geo} \leq 1$	1	Uncontaminated to moderately contaminated
$1 < I_{geo} \leq 2$	2	Moderately contaminated

Igeo value	Class	Soil quality
$2 < \text{Igeo} \leq 3$	3	Moderately to heavily contaminated
$3 < \text{Igeo} \leq 4$	4	Heavily contaminated
$4 < \text{Igeo} \leq 5$	5	Heavily to extremely contaminated
$\text{Igeo} > 5$	6	Extremely contaminated

A numeric illustration clarifies the scale. Suppose a soil contains 2.5 mg kg^{-1} of cadmium against a regional background of 0.5 mg kg^{-1} . Then $\text{Igeo} = \log_2[2.5 / (1.5 \times 0.5)] = \log_2(3.33) \approx 1.74$, placing the sample in Class 3, moderately to heavily contaminated. Doubling the concentration to 5 mg kg^{-1} raises Igeo by exactly one unit to about 2.74, still within the same upper class boundary, which demonstrates how the logarithmic scale compresses large concentration ranges into a compact, interpretable index.

5.3 Enrichment factor

The enrichment factor (EF) corrects for natural variation in grain size and mineral abundance by normalising the target element to a conservative reference element, typically aluminium, iron, or titanium, in both sample and background:

$$EF = [C(\text{element})/C(\text{reference})]_{\text{sample}} / [C(\text{element})/C(\text{reference})]_{\text{background}}$$

By dividing out a lithogenic carrier, the EF isolates the fraction of an element that cannot be attributed to ordinary mineralogical variation, which makes it particularly useful for separating geogenic from anthropogenic contributions. Sutherland (2000) proposed a five-category interpretation (Table 2). An important caution is that the EF is sensitive to the choice of reference

element and to any anthropogenic perturbation of that reference; iron, for instance, is unsuitable where iron-bearing dust is itself a contaminant.

Table 2. Sutherland (2000) enrichment factor categories.

EF value	Enrichment category
EF < 2	Deficiency to minimal enrichment
$2 \leq EF < 5$	Moderate enrichment
$5 \leq EF < 20$	Significant enrichment
$20 \leq EF < 40$	Very high enrichment
$EF \geq 40$	Extremely high enrichment

6. Integrated Multi-Element Indices

Single-element indices describe one element at a time. Integrated indices summarise the cumulative burden of several elements at a site, which is essential for ranking locations and communicating an overall condition. Four are in common use.

6.1 Pollution load index

Tomlinson et al. (1980) defined the pollution load index (PLI) as the geometric mean of the contamination factors of n elements at a site:

$$PLI = (CF_1 \times CF_2 \times \dots \times CF_n)^{(1/n)}$$

A PLI value of 1 indicates that metals are present only at background level; values above 1 indicate progressive deterioration. The geometric mean prevents a single extreme element from dominating the index, which is both a strength, in that it summarises

overall condition, and a limitation, in that it can mask a single hazardous element.

6.2 Nemerow integrated pollution index

The Nemerow integrated pollution index combines the mean and the maximum of the single-element contamination factors so that the most polluted element is given explicit weight:

$$PN = \sqrt{\{ [(C\bar{F})^2 + (CF_{max})^2] / 2 \}}$$

where $C\bar{F}$ is the average and CF_{max} the maximum contamination factor at the site. Because it retains the worst-case element, the Nemerow index complements the PLI and is often reported alongside it.

6.3 Degree of contamination

Håkanson (1980) defined the degree of contamination (C_d) as the sum of contamination factors across the elements considered:

$$C_d = \sum CF_i \quad (i = 1 \dots n)$$

Because the absolute value of C_d depends on the number of elements summed, a modified degree of contamination, which divides the sum by the number of elements, is frequently used to allow comparison among studies that consider different element suites.

6.4 Potential ecological risk index

Håkanson (1980) also introduced the potential ecological risk index, which weights each element by a toxic-response factor that reflects its ecological and toxicological hazard. The single-element risk factor is

$$E_r^i = T_r^i \times CF_i$$

where T_r^i is the toxic-response factor (for example, commonly cited values increase from zinc and chromium through

copper, lead, and nickel to cadmium and mercury, reflecting their relative toxicity). The summed potential ecological risk index is

$$RI = \sum E_r^i$$

The risk index therefore integrates both the degree of enrichment and the intrinsic toxicity of each element, which makes it more ecologically meaningful than indices based on concentration alone. Table 3 gives the standard interpretive bands for the single-element factor and the summed index.

Table 3. *Håkanson (1980) ecological risk classification.*

E _r (single element)	RI (summed)	Ecological risk
E _r < 40	RI < 150	Low risk
40 ≤ E _r < 80	150 ≤ RI < 300	Moderate risk
80 ≤ E _r < 160	300 ≤ RI < 600	Considerable risk
160 ≤ E _r < 320	RI ≥ 600	High / very high risk
E _r ≥ 320	—	Very high risk

6.5 Composite soil quality indices

Beyond contamination-specific indices, composite soil quality indices (CSQI) aggregate physicochemical fertility indicators (pH, electrical conductivity, organic matter, total nitrogen and carbon) with contamination measures into a single scaled score, often on a 0–100 range. Such indices are useful for communicating an integrated condition to non-specialist stakeholders, but the weighting and scaling scheme must be transparent because the final score is sensitive to those subjective choices.

6.6 Choosing and reporting indices

No single index is sufficient, and reporting many without interpretation is equally unhelpful. A defensible assessment pairs a single-element index that preserves elementwise detail, usually the geoaccumulation index or enrichment factor, with an integrated index that summarises the site, usually the pollution load index complemented by a worst-case measure such as the Nemerow index, and an ecological-risk index that incorporates toxicity. The geoaccumulation index and enrichment factor indicate how enriched each element is relative to background; the pollution load and Nemerow indices indicate how degraded each site is overall; and the potential ecological risk index indicates how hazardous the contamination is ecologically. Reporting one index from each of these complementary families, with the background and toxic-response factors stated, gives a complete and reproducible picture without redundancy.

7. Multivariate Source Apportionment

Indices quantify the extent of contamination; multivariate statistics help explain its sources. The premise is that elements sharing a common source covary across samples, so the correlation structure of a multi-element dataset encodes information about source groupings.

7.1 Correlation analysis

A correlation matrix is the natural first step. Strong positive correlations among a subset of elements suggest a shared origin or a common geochemical carrier, while the absence of correlation argues for independent sources. Correlations must be interpreted cautiously, however, because spurious associations can arise from grain-size covariation or from closure effects in compositional data.

7.2 Principal component and factor analysis

Principal component analysis (PCA), often with varimax rotation, reduces many correlated elements to a few latent factors that can be interpreted as sources. A factor loading heavily on lead, cadmium, and beryllium together with electrical conductivity and petroleum hydrocarbons, for example, is readily interpreted as a combined refinery-and-vehicular signature, whereas a factor loading on nickel, chromium, copper, and zinc points to a mixed geogenic-anthropogenic origin, and a factor dominated by organic matter, total nitrogen, and organic carbon reflects soil fertility rather than contamination. The proportion of variance explained by each component indicates its relative importance.

7.3 Hierarchical cluster analysis

Hierarchical cluster analysis (HCA) groups either elements or sampling sites by similarity, producing a dendrogram. Clustering of elements corroborates the factor structure from PCA; clustering of sites delineates spatial contamination domains, for example separating an industrial core from a low-contamination periphery. Used together, PCA and HCA provide convergent rather than independent evidence, and they are most persuasive when their groupings agree with the index-based and geochemical interpretation rather than standing alone.

7.4 Quantitative receptor models

Beyond the exploratory grouping provided by principal component and cluster analysis, receptor models attempt to apportion the measured concentration of each element quantitatively among a set of sources. Absolute principal component scores combined with multiple linear regression (APCS-MLR) translate rotated factor scores into the mass contribution of each factor to each element, while positive matrix factorisation (PMF) decomposes the data into non-negative source profiles and contributions without requiring prior knowledge of the sources. These methods move the

analysis from a qualitative statement that certain elements share a factor to a quantitative estimate of, for example, the percentage of soil lead attributable to a refinery-vehicular source versus the lithogenic background. Their outputs are model-dependent and require careful diagnostic checking, but where the data support them they substantially strengthen a source-apportionment argument and provide the apportioned inputs that some exposure models can use.

8. Spatial Structure and Geostatistical Mapping

Contamination indices computed point by point become far more useful when their spatial structure is modelled, because management decisions are made over areas rather than at individual points. Two interpolation approaches dominate practice, and the difference between them is consequential.

8.1 Deterministic interpolation

Inverse distance weighting (IDW) estimates a value at an unsampled location as a distance-weighted average of nearby measurements. It is simple, makes no statistical assumptions, and is adequate for a quick visualisation, but it provides no measure of estimation uncertainty and tends to produce characteristic bull's-eye artefacts around isolated extreme values. For a definitive assessment, its lack of an uncertainty estimate is a serious limitation.

8.2 Geostatistical interpolation

Ordinary kriging treats the measured concentrations as a realisation of a spatially correlated random field and uses the empirical semivariogram, which quantifies how dissimilarity between points grows with separation distance, to derive optimal interpolation weights. Fitting a model to the semivariogram, characterised by its nugget, sill, and range, captures the spatial autocorrelation of the data, and kriging then yields both a best linear unbiased prediction and a kriging variance that maps estimation

uncertainty. The latter is its decisive advantage: it tells the analyst where the interpolated surface is well constrained and where additional sampling would most reduce uncertainty.

Reliable variogram estimation requires an adequate number of sample pairs across the range of distances, which is why the spatial design of Section 3.4 matters. With small datasets, the variogram is poorly constrained and the case for kriging over a simpler method weakens; cross-validation, in which each point is removed in turn and predicted from the others, provides a quantitative check on model performance through statistics such as the mean and root-mean-square standardised prediction error. Where the variogram cannot be modelled credibly, a deterministic method should be used with an explicit acknowledgement of its limitations rather than fitting an unsupported geostatistical model.

8.3 Mapping indices rather than concentrations

Interpolating index values, or the contamination and enrichment factors of individual elements, rather than raw concentrations, produces maps that are directly interpretable in terms of contamination class and that delineate management zones. Such maps are most persuasive when the spatial patterns of independently derived indices, and of the multivariate factor scores from Section 7, coincide, since convergent spatial evidence is harder to attribute to artefact than any single interpolated surface.

9. A Worked Semi-Arid Industrial Example

The following synthesis illustrates the workflow for a representative semi-arid landscape of approximately 350 km² surrounding a petroleum refinery, sampled at 22 surface sites during a single dry-season campaign and analysed for seventeen metals plus total petroleum hydrocarbons (TPH) along with physicochemical fertility parameters. The soils are alkaline (mean pH $\approx$ 8.3) and

saline-influenced (mean electrical conductivity $\approx 4.8 \text{ dS m}^{-1}$), with modest organic matter, conditions typical of the region.

Applying the geoaccumulation index against regionally appropriate background values, rather than a global crustal average, yields a clear three-way separation. Beryllium reaches a mean Igeo consistent with the heavily-contaminated class, and cadmium and lead fall in the moderately-to-heavily contaminated range, while nickel, chromium, and cobalt return negative Igeo values that place them firmly in the lithogenic, practically-uncontaminated category. This pattern is the heart of the interpretation: the enriched elements are those expected from combustion, vehicular, and refining sources, and the unenriched elements are those expected to track parent-material mineralogy.

Table 4. Illustrative geoaccumulation results for the worked example (regional background).

Element	Mean Igeo	Müller class	Interpretation
Be	≈ 3.6	5	Heavily–extremely contaminated
Cd	≈ 1.7	3	Moderately–heavily contaminated
Pb	≈ 1.0	3	Moderately–heavily contaminated
Mo	≈ 0.7	2	Moderately contaminated
Ni	≈ -1.7	0	Lithogenic, uncontaminated
Cr	≈ -2.0	0	Lithogenic, uncontaminated
Co	≈ -1.5	0	Lithogenic, uncontaminated

Spatially, the enriched elements concentrate in an industrial cluster of sites adjacent to the refinery, while peripheral sites return low contamination factors for lead and cadmium. A composite soil quality index computed across the site set ranges from roughly 42 to 81, with a mean near 61, ranking the industrial core as the most degraded and the periphery as comparatively intact.

The contamination factors implied by these geoaccumulation results follow directly from the index definitions, since $CF = 1.5 \times 2^{I_{geo}}$. Table 5 lists the derived factors and the corresponding Håkanson contamination class. Beryllium emerges as very highly contaminated, cadmium and lead as considerable to moderate, and the lithogenic elements as low, exactly the separation expected if the enriched elements are anthropogenic and the others inherited from the parent material.

Table 5. Contamination factors derived from the worked-example geoaccumulation values.

Element	Implied CF	Håkanson class
Be	≈ 17.7	Very high ($CF \geq 6$)
Cd	≈ 4.7	Considerable (3–6)
Pb	≈ 3.1	Considerable (3–6)
Mo	≈ 2.5	Moderate (1–3)
Ni	≈ 0.45	Low ($CF < 1$)
Cr	≈ 0.37	Low ($CF < 1$)
Co	≈ 0.55	Low ($CF < 1$)

Multivariate analysis reinforces this reading. A principal component analysis resolves three interpretable factors. The first and largest associates lead, cadmium, and beryllium with electrical conductivity and petroleum hydrocarbons, a refinery-vehicular signature; the second groups nickel, chromium, copper, and zinc, a mixed geogenic-anthropogenic association; and the third loads on organic matter, total nitrogen, and organic carbon, a fertility dimension unrelated to contamination. Cluster analysis of the sites separates the industrial core from the periphery in agreement with the index maps. No single line of evidence is decisive on its own, but the convergence of geoaccumulation classes, enrichment factors, spatial pattern, and multivariate structure makes the anthropogenic attribution of beryllium, cadmium, and lead robust, and the lithogenic attribution of nickel, chromium, and cobalt equally so.

Two methodological points are worth drawing from this example. First, had a regulatory guideline been used in place of the regional geochemical background, several lithogenic elements would have appeared spuriously enriched and the clean separation of sources would have been lost; the background choice is what makes the interpretation legible. Second, the agreement between the geostatistical maps and the multivariate factor scores is what elevates the conclusion from plausible to robust, illustrating the value of demanding convergent evidence rather than relying on any one tool.

Enrichment factors, computed with a conservative lithogenic normaliser, tell the same story from a different direction. The elements that the geoaccumulation index flags as contaminated also return high enrichment factors once natural grain-size and mineralogical variation is divided out, while the lithogenic elements cluster near unity, the value expected when an element is present only in its natural proportion to the reference. That two indices built on different normalisation logics, one against a scaled background

and one against an internal reference element, agree on the same source assignment is itself a form of convergent evidence and increases confidence that the pattern is real rather than an artefact of either method.

10. Limitations and Good Practice

Indices are only as sound as the background. State the reference, its source, and whether it is global, regional, or statistically derived, and where feasible report results against more than one background.

Match the index to the digestion. Geoaccumulation, enrichment, and contamination factors are defined on total or pseudo-total inventories and must not be presented as statements about bioavailability.

Single-campaign, dry-season sampling captures a snapshot. Seasonal and temporal variability, and the absence of subsurface profiling, are genuine constraints that should be acknowledged rather than glossed over.

Geometric-mean indices such as the PLI can mask a single hazardous element; pair them with an index that retains the worst case, such as the Nemerow index or the single-element ecological risk factor.

Multivariate factors are statistical constructs, not measured sources. Treat PCA and HCA as corroborating evidence that must agree with the geochemical and spatial interpretation, not as independent proof.

Report uncertainty. Detection limits, certified-reference recoveries, and replicate precision determine how many contamination classes a value can credibly span.

11. Conclusions and Link to Health Risk Assessment

A credible geochemical assessment of semi-arid industrial soils is a chain of defensible decisions rather than a single calculation. The sampling fraction and digestion fix what is measured; the background fixes what the measurement means; the single-element and integrated indices translate concentrations into interpretable enrichment, contamination, and ecological-risk classes; and multivariate analysis supplies convergent evidence about source. When these steps agree, as in the worked example where beryllium, cadmium, and lead emerge consistently as anthropogenic while nickel, chromium, and cobalt remain lithogenic, the resulting interpretation is robust to reasonable changes in method (Dilekoglu et.al. 2026).

The assessment also carries management implications. Because the enriched elements in the worked example are spatially concentrated in an industrial core, remediation and monitoring resources can be targeted rather than dispersed across the whole landscape, and the source apportionment indicates which emission pathways, refining and vehicular, would most reduce future loading if controlled. The kriging variance maps identify where additional sampling would most improve confidence before any intervention. Equally important, the recognition that nickel, chromium, and cobalt are lithogenic prevents the costly error of treating naturally elevated metals as pollution requiring remediation. Distinguishing what must be managed from what is merely inherited is one of the most practically valuable outcomes of a rigorous index-based assessment.

Ecological-risk and contamination indices describe the condition of the soil itself. They do not, however, quantify the consequences of that condition for the people who live and work on the land. Translating measured soil concentrations into estimates of human exposure and harm requires a separate, exposure-based framework, the human health risk assessment built on the United States Environmental Protection Agency's Risk Assessment

Guidance for Superfund (USEPA, 1989). That framework, with its hazard quotients, hazard indices, and incremental lifetime cancer risks for adult and child receptors across ingestion, dermal, and inhalation pathways, is the subject of the next chapter.

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CHAPTER 8

SMART PHENOTYPING AND IMAGE PROCESSING-BASED PLANT ANALYSIS IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

SUAT CUN¹

Introduction

The increasing global population, the growing impacts of climate change, and the necessity for sustainable agricultural production have created an urgent need for the development of faster, more precise, and high-throughput measurement techniques in plant breeding and agronomic research. Conventional phenotyping methods are largely based on the manual assessment of plant traits, making them labor-intensive, time-consuming, and prone to low repeatability, while also being highly susceptible to environmental variability. Consequently, these limitations restrict the accurate evaluation of genotype $\times$ environment interactions, particularly in large-scale breeding programs (Furbank and Tester, 2011; White et al., 2012).

In recent years, the concept of “smart phenotyping” has emerged as a next-generation approach that enables the rapid,

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automated, and highly accurate analysis of plant phenotypes. This approach facilitates the non-invasive assessment of plant traits through the integration of high-resolution imaging systems, remote sensing technologies, sensor-based measurements, and artificial intelligence algorithms (Araus and Cairns, 2014; Tardieu et al., 2017). In particular, high-throughput phenotyping platforms are capable of monitoring plant growth dynamics in real time and enabling large-scale analyses based on extensive datasets (Fahlgren et al., 2015).

Image-processing-based plant phenotyping systems allow the precise quantification of numerous parameters, including leaf area, vegetation indices, chlorophyll distribution, water stress indicators, and growth rates. In particular, multispectral, hyperspectral, and thermal imaging techniques have become essential tools for assessing the physiological status of plants (Li et al., 2014; Munns and James, 2003). By enabling the early detection of plant stress, these technologies offer significant potential for minimizing yield losses and improving crop management practices (Jones and Vaughan, 2010; Mulla, 2013).

Cotton (*Gossypium hirsutum* L.) is one of the most strategically important industrial crops in global agriculture due to its fiber quality and substantial economic value. Furthermore, its sensitivity to abiotic stresses such as drought, salinity, and nutrient deficiencies has made it an important model species in phenotyping research. In cotton, image-processing-based phenotyping approaches are increasingly being employed to assess traits related to nitrogen use efficiency, leaf development, and photosynthetic activity (Zhao et al., 2010). Compared with conventional measurement techniques, these approaches provide faster, more objective, and highly reproducible results.

In this context, smart phenotyping and image-processing-based analytical techniques are driving a transformative shift in

modern plant breeding and agricultural research. These technologies play a critical role in evaluating genotype performance, understanding stress tolerance mechanisms, and developing yield-enhancing strategies in strategically important industrial crops such as cotton (Araus et al., 2018; Tardieu et al., 2017).

Phenotyping in Cotton

Phenotyping in cotton involves the evaluation of a wide range of morphological, physiological, and biochemical traits that determine the agronomic performance of the plant. These traits include parameters such as plant height, leaf area index, chlorophyll content, biomass production, boll number, lint yield, and fiber quality. In addition, since cotton is a sensitive industrial crop to abiotic stresses, physiological responses to water stress, salinity, and nutrient deficiencies also play a significant role in phenotyping studies (Pauli et al., 2016).

Traditional cotton phenotyping methods are largely based on manual measurements; therefore, they are time-consuming and present scalability limitations in large-scale breeding programs. Moreover, human-induced measurement errors and environmental variability reduce the reproducibility of the data. For this reason, phenotyping processes in cotton have long been considered a limiting factor for genetic gain (Araus and Cairns, 2014).

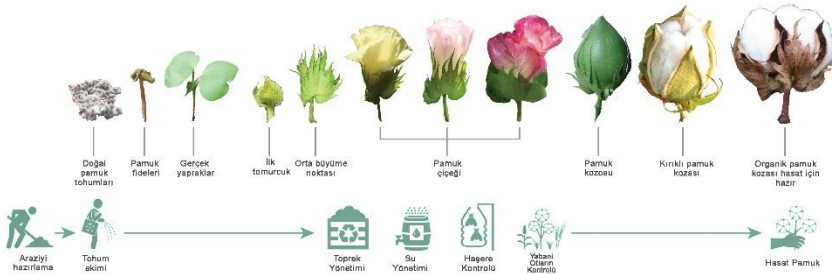


Figure 1. Developmental stages of the cotton plant from seed to harvest.

In recent years, the concept of phenotyping has undergone a significant transformation with the development of high-throughput phenotyping technologies. This shift has enabled cotton phenotyping processes to evolve from being based solely on visual observations into a more digital framework supported by image processing, remote sensing, and sensor-based measurement systems (Fahlgren et al., 2015; Tardieu et al., 2017). In particular, multispectral and thermal imaging techniques allow for the non-invasive determination of physiological parameters such as water status and photosynthetic activity in cotton plants (Li et al., 2014; Jones and Vaughan, 2010).

Phenotyping in cotton also plays a critical role in accelerating breeding programs. High-throughput phenotyping systems enable the evaluation of the responses of different genotypes to environmental stress conditions in a shorter time and with higher accuracy, thereby accelerating the selection process of superior genotypes (Araus et al., 2018). This provides significant advantages, particularly in the assessment of complex traits such as drought tolerance and nitrogen use efficiency. This approach represents not only the measurement of plant traits but also the integrated analysis of data for a holistic evaluation of plant performance. Today, phenotyping has become an indispensable tool in cotton breeding for accelerating genetic gain and developing sustainable production systems (Singh et al., 2016; Tardieu et al., 2017).

Image Processing and Computer Vision Applications

Image-based plant phenotyping is a multidisciplinary approach that aims to automatically extract plant traits from high-resolution images and represents one of the key components of digital transformation in agricultural systems. Compared to conventional manual measurement techniques, this method offers higher scalability and provides significant advantages in terms of

time and labor efficiency, particularly in large-scale breeding programs. Phenotyping processes have evolved not only in data acquisition but also in data interpretation toward artificial intelligence–assisted systems (Fiorani and Schurr, 2013; Araus and Cairns, 2014).

Computer vision algorithms enable the automatic extraction of morphological and physiological traits from plant images. In particular, segmentation-based deep learning models (such as U-Net and Mask R-CNN) provide high accuracy in separating plant organs from the background and allow precise measurement of traits such as leaf area, stem geometry, and canopy structure. These approaches produce more robust results against variations in lighting conditions and environmental noise compared to traditional image processing methods (Kamilaris and Prenafeta-Boldú, 2018).

The integration of deep learning-based models into agricultural phenotyping has entered a new phase, particularly with the use of convolutional neural networks (CNNs) as well as Vision Transformer (ViT) architectures. These models learn features directly from plant images and demonstrate high performance in tasks such as disease detection, stress classification, and biomass estimation. In recent years, transformer-based approaches have emerged as a strong alternative to CNN-based models, especially in large-scale datasets (Dosovitskiy et al., 2020).

Image-based phenotyping applications in cotton have become an important tool, particularly for monitoring morphological changes associated with high temperature, drought, and nitrogen stress. Data obtained through UAV (unmanned aerial vehicle)-based imaging systems enable the temporal monitoring of vegetation indices (NDVI), canopy development, and boll formation dynamics in cotton fields. This approach allows for earlier and more objective assessment of the stress tolerance capacity of different genotypes (Thorp et al., 2015).

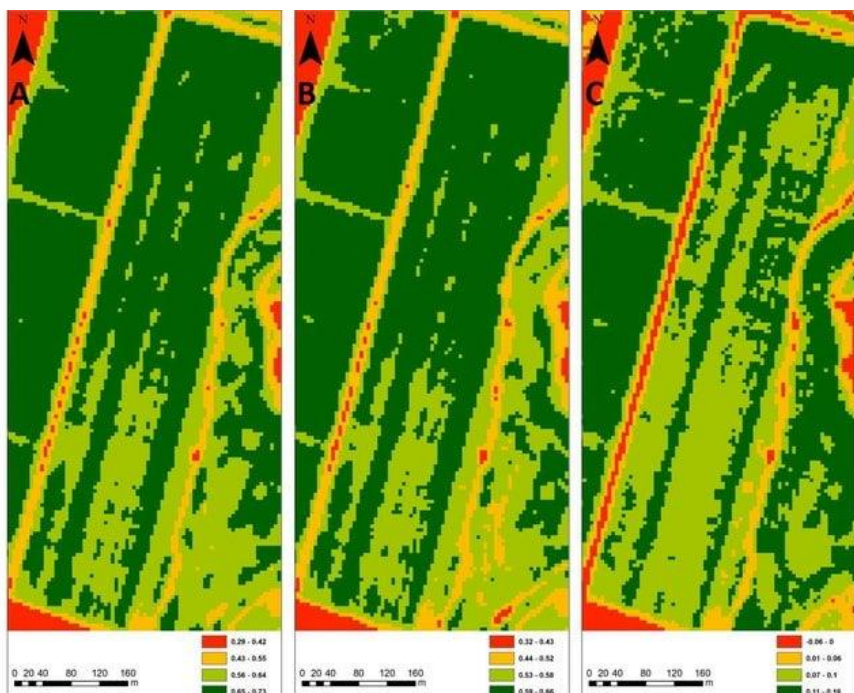


Figure 2. Satellite image-based vegetation indices NDVI (A), GNDVI (B), and GRVI (C) (Chen et al., 2019)

Multispectral and hyperspectral imaging systems extend beyond the visible spectrum, enabling the analysis of plant physiology using near-infrared (NIR) and short-wave infrared (SWIR) bands. These techniques offer high sensitivity, particularly for the early detection of abiotic stresses such as nitrogen deficiency and water stress. In recent years, the integration of spectral data with deep learning models has promoted a “data-driven physiology” approach in phenotyping (Feng et al., 2020; Furbank et al., 2021).

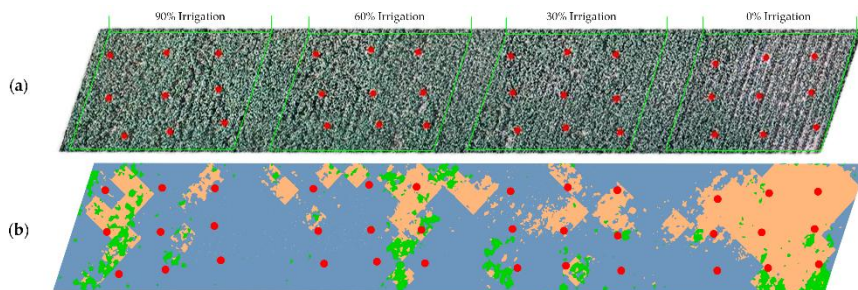


Figure 3. Original UAV (UAS) image of a cotton field (Esirige et al., 2026).

UAV (Drone) and Hyperspectral Imaging

Unmanned aerial vehicles (UAVs; drones) and hyperspectral imaging technologies have brought about a significant transformation in plant phenotyping studies by enabling the acquisition of high-resolution, rapid, and repeatable data in modern agriculture. In particular, the limitations of manual measurement methods in large agricultural areas have placed UAV-based remote sensing systems at the center of phenotyping research. These systems offer cost-effective operation along with high spatial and temporal resolution, allowing the dynamic monitoring of plant development (Yang et al., 2017; Maes and Steppe, 2019).

UAV-based imaging systems are typically equipped with RGB, multispectral, and thermal sensors and are widely used for the determination of phenotypic traits such as vegetation indices (NDVI, GNDVI), canopy cover, plant height, and biomass estimation. These technologies enable temporal data acquisition throughout the growing season, allowing a more detailed analysis of genotype responses to environmental conditions. In this context, UAV systems have become one of the most important field-based components of high-throughput phenotyping platforms (Yang et al., 2017; Roth et al., 2018).

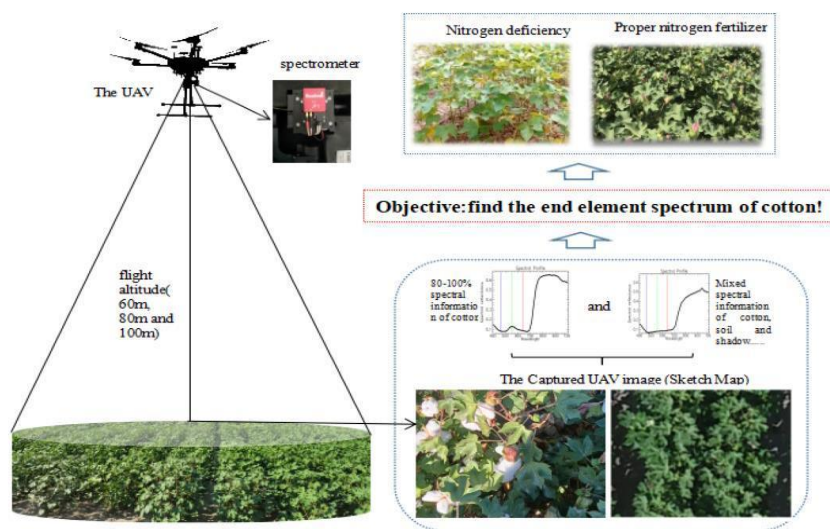


Figure 4. (a) UAV flight/experimental setup and (b) spectral reflectance curves or false-color hyperspectral map (Yin et al., 2022).

Hyperspectral imaging, on the other hand, provides much more detailed information on plant physiology by measuring reflected light from plants across very narrow spectral bands. This technology plays a critical role, particularly in the early detection of abiotic and biotic stress factors such as water stress, nitrogen deficiency, and disease. Hyperspectral data reveal the “spectral signature” of plants, enabling the detection of physiological changes that cannot be observed in visible light (Mahlein, 2016; Feng et al., 2020).

In industrial crops such as cotton, these systems provide significant advantages in yield prediction, stress mapping, and genotype performance comparison. When high-resolution UAV data are combined with machine learning algorithms, prediction accuracy significantly improves (Feng et al., 2020). However, hyperspectral imaging requires advanced data processing techniques due to its

large data volume and high computational demands. Therefore, deep learning-based dimensionality reduction and feature selection methods are increasingly used in hyperspectral data analysis. These approaches help extract the most relevant information from spectral data, contributing to more efficient phenotyping processes (Paoletti et al., 2019).

Machine Learning and Data Analysis

Machine learning is a subfield of artificial intelligence that automates prediction, classification, and decision-making processes by extracting meaningful patterns from large and complex datasets. In the context of agricultural phenotyping, machine learning plays a critical role, particularly in the analysis of image-based and sensor-based data. While traditional statistical methods are often insufficient for analyzing high-dimensional plant-derived datasets, machine learning algorithms enable more accurate inference from such data (Liakos et al., 2018; Chlingaryan et al., 2018). In particular, algorithms such as Random Forest, Support Vector Machine (SVM), and Gradient Boosting have demonstrated high performance in agricultural data analysis (Kamilaris and Prenafeta-Boldú, 2018). Singh et al. (2016) and van Dijk et al. (2021) emphasized that machine learning has become an important tool for modeling genotype responses to environmental stresses.

In cotton, machine learning applications are widely used in areas such as yield prediction, stress classification, nitrogen use efficiency analysis, and growth modeling. The integration of UAV- and sensor-based data with machine learning algorithms enables more accurate modeling of genotype $\times$ environment interactions. This approach facilitates the earlier and more reliable selection of superior genotypes in breeding programs (Aierken et al., 2024). Furthermore, the integration of big data analytics and machine learning has accelerated the development of decision support systems in agricultural systems. These systems not only analyze

historical data but also predict future plant performance, thereby improving risk management and resource use optimization in agricultural production (Chlingaryan et al., 2018).

Yield Prediction and Decision Support Systems

Yield prediction and decision support systems represent one of the most critical components of the digital transformation in modern agriculture. These systems enable forward-looking predictions in crop production, optimize resource use, reduce risks, and place production planning on a more scientific basis. In particular, yield prediction is of strategic importance in economically valuable industrial crops such as cotton, both for producers' decisions and breeding programs (Chlingaryan et al., 2018).

Traditional yield prediction methods are largely based on field observations, sampling, and statistical regression models. However, these approaches are often insufficient to fully capture genotype $\times$ environment interactions and typically provide limited spatial and temporal resolution. Therefore, data-driven approaches integrating remote sensing, UAV imaging, and sensor data have gained significant importance (Aierken et al., 2024).

Machine learning-based yield prediction models enable the analysis of multidimensional datasets and produce more accurate predictions. These models can estimate cotton yield by using variables such as vegetation indices (NDVI, EVI), leaf area, biomass, temperature, and water stress as inputs. In particular, Random Forest, Gradient Boosting, and deep learning-based regression models are widely used in agricultural yield prediction (Liakos et al., 2018).

Decision support systems (DSS) are digital platforms that integrate outputs from yield prediction models into agricultural decision-making processes. These systems guide farmers and

researchers in critical decisions such as planting time, fertilization strategies, irrigation scheduling, and harvest timing. AI-based DSS analyze historical datasets to provide future-oriented recommendations and reduce uncertainty in agricultural production (Kamilaris and Prenafeta-Boldú, 2018).

In cotton production, decision support systems contribute significantly to areas such as nitrogen management, irrigation optimization, and stress management. For example, image-based phenotyping data can be used to detect nutrient deficiencies at early stages, enabling optimized intervention timing. This provides substantial benefits in terms of both yield improvement and reduced input costs (Aierken et al., 2024). Moreover, the development of big data analytics and cloud-based agricultural platforms has made decision support systems more scalable. These systems can integrate data from multiple sources (UAV, sensors, meteorological data), perform real-time analysis, and provide instant recommendations to farmers. Thus, the transition from reactive management to proactive and predictive management in agriculture has been significantly accelerated (Chlingaryan et al., 2018).

Conclusion

The present study demonstrates that phenotyping processes in modern agriculture, particularly in cotton production, are undergoing a rapid transformation from traditional methods toward digital and data-driven approaches. Increasing global population, climate change, and the demand for sustainable production necessitate the development of faster, more accurate, and scalable measurement systems in plant breeding and agronomic research.

Image-based phenotyping and computer vision techniques have significantly reduced the limitations of manual measurements by enabling the high-accuracy and non-invasive assessment of plant traits. In particular, multispectral and hyperspectral imaging systems

contribute critically to stress management and yield optimization by allowing the early detection of the physiological status of plants. The widespread adoption of UAV-based systems has further enabled high-resolution and temporal data acquisition at the field scale, improving the analysis of genotype $\times$ environment interactions.

The integration of machine learning and artificial intelligence algorithms with these data sources has accelerated the transition from descriptive analysis to predictive modeling in agricultural systems. In particular, yield prediction and stress classification models enhance breeding efficiency by supporting faster and more reliable genotype selection in cotton breeding programs.

In conclusion, smart phenotyping and digital agriculture technologies provide a powerful toolkit for increasing productivity, understanding stress tolerance mechanisms, and developing production systems adapted to environmental variability in cotton and other industrial crops. With further advancements in these technologies, data integration, automation, and AI-driven analytics are expected to play a central role in future agricultural research.

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CHAPTER 9

MICROALGAE AS BIOFERTILIZERS AND BIOSTIMULANTS: NEW TECHNOLOGIES FOR INTEGRATED AGRICULTURE

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EMRAH RAMAZANOĞLU²

Introduction

As a dynamic component of terrestrial environments, soil plays a fundamental role in sustaining ecosystem functions and supporting the long-term sustainability of agricultural systems. The effective functioning of soil depends on interrelated properties such as soil health, soil quality, and soil fertility. Healthy soil is a dynamic system that not only maintains water-related characteristics and plant productivity but also performs numerous ecological functions by regulating nutrient cycling and organic matter decomposition (Sen et al., 2025). This characteristic is closely associated not only with physical and chemical parameters but also with microbial activity and enzymatic processes. Although soil health is an inherent property of soil, it can undergo significant changes as a result of anthropogenic activities and land-use practices (Tahat et al., 2020).

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Furthermore, soil fertility is defined not only as the capacity to supply essential nutrients required for plant growth but also as the ability to provide sufficient soil water needed by plants (FAO, 1976; Havlin et al., 2016). This capacity is determined by the combined influence of numerous physical, chemical, and biological factors (Cheik and Jouquet, 2020). Therefore, the maintenance and improvement of soil health constitute the foundation of sustainable agricultural production, ensuring both the continuity of productivity and the preservation of ecosystem stability.

Increasing agricultural production while preserving environmental quality is one of the most significant challenges facing the scientific community today (Murata et al., 2021; Chiaiese et al., 2018). The increase in global food demand has led to the intensification of agricultural production, which has in turn heightened the importance of ensuring the timely and sufficient supply of essential soil nutrients. As agricultural systems become more intensive, the availability and rapid replenishment of key soil nutrients have become critical factors for maintaining productivity and crop performance (Kour et al., 2020; Chittora et al., 2020). In this context, agricultural systems have become heavily dependent on chemical fertilizers to satisfy production demands (Kumar et al., 2018). While NPK (nitrogen, phosphorus, and potassium) chemical fertilizers may boost crop yields over the short term, long-term use has been associated with harmful effects on soil health (Yin et al., 2018).

These chemical fertilizers contribute to ecosystem destabilization by causing acidification through oxidation processes, loss of basic cations via ion exchange mechanisms, and disruption of soil biochemistry through nitrification processes (Cai et al., 2019). As a result, soil structure degradation, a decline in microbial diversity, and weakening of natural nutrient cycles collectively lead to reduced productivity and the emergence of crops with lower

nutritional quality (Mukherjee et al., 2016; Wang et al., 2020; Kumar et al., 2018). In addition, excessive use of chemical fertilizers induces severe environmental problems such as eutrophication, leading to degradation of aquatic ecosystems through surface runoff and leaching processes (Cui et al., 2020; Huang et al., 2017). On a global scale, the fact that approximately 9.4–17.1% of greenhouse gas emissions are attributable to chemical fertilizers indicates that this issue is not only agronomic but also has a significant climatic dimension.

In response to these environmental and agronomic challenges, biofertilizers have emerged as an environmentally friendly alternative. Biofertilizers can improve soil fertility, increase nutrient availability, and support plant growth and development (Nayak et al., 2019; Osorio-Reyes et al., 2023; Kumar et al., 2022). By contributing to the reduction of chemical inputs, they play an important and strategic role in maintaining and improving soil health.

Among biofertilizer formulations based on photosynthetic organisms, microalgae have an important and distinctive role due to their high biomass production potential and rich metabolite composition (Guo et al., 2020; Cakirsoy et al., 2022). Microalgae are used in various fields such as biofuel production, food industries, and biofertilizer applications due to their capacity to synthesize diverse bioactive metabolites, including polysaccharides, proteins, phytohormones, antioxidant compounds, lipids, and reducing sugars (Ronga et al., 2019; Gonçalves, 2021).

Additionally, they are capable of synthesizing growth regulators such as abscisic acid, auxins, gibberellins, ethylene, and cytokinins, as well as macronutrients including nitrogen, phosphorus, and carbon (Van T Do et al., 2021; Gao et al., 2023; Maltsev et al., 2023). The release of these compounds following cell lysis makes microalgae a valuable source for use as fertilizers and

biostimulants in agriculture (Ng and Chew, 2024). These characteristics position microalgae as a biotechnological tool that can serve as an alternative or complementary input to chemical fertilizers in sustainable agricultural systems.

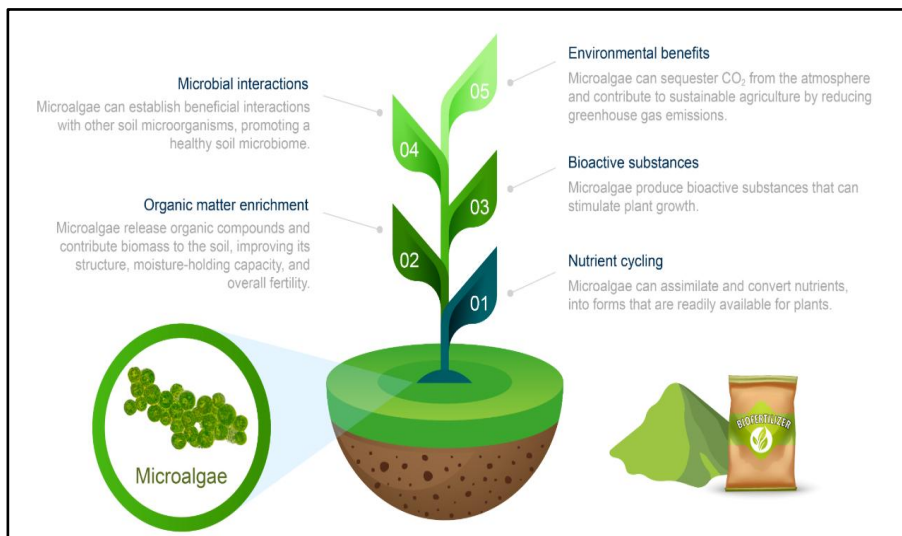


Figure 1. Various Benefits of Microalgae Utilization as Biofertilizers (Gonçalves et al., 2023)

Biological and Ecological Properties of Algae

Macroalgae and microalgae are generally classified under the collective term “algae” and constitute a large and diverse group of organisms predominantly found in aquatic environments. To date, more than 156,000 eukaryotic algal species have been identified, indicating a high level of biological diversity within this group (Hughes et al., 2021). Owing to their photosynthetic capabilities, these organisms play an important and strategic role in both ecosystem functioning and biotechnological applications.

Macroalgae are multicellular organisms found in both freshwater and marine ecosystems and are primarily classified into green algae (Chlorophyta), brown algae (Phaeophyta), and red algae

(Rhodophyta) (Øverland et al., 2019). They play an important ecological role by contributing to oxygen production in marine environments and functioning as primary producers within marine food webs (Chan et al., 2006). In addition, macroalgae are harvested from natural environments as well as cultivated under controlled conditions, and are widely consumed as traditional food sources, particularly in Asian countries. At the industrial scale, they are widely used in various applications, including food, cosmetics, pharmaceuticals, and agricultural product formulations.

Microalgae are photoautotrophic microorganisms characterized by rapid growth and a high capacity to adapt to a wide range of environmental conditions (Ortiz-Moreno et al., 2019). This group includes not only eukaryotic microalgae but also prokaryotic cyanobacteria. Their simple unicellular structure, high photosynthetic efficiency, ability to grow heterotrophically, tolerance to domestic and industrial wastewater, suitability for metabolic engineering, and potential to produce valuable by-products make microalgae highly attractive for biotechnological applications (Chiaiese et al., 2018). In addition, algae are becoming increasingly important in agriculture due to their growing use as biofertilizers (Grzesik et al., 2017).

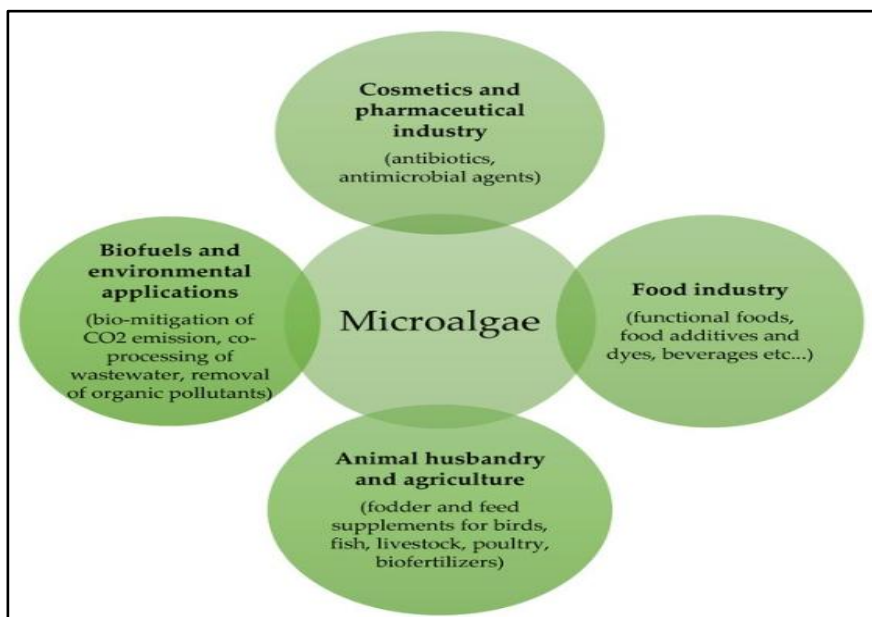


Figure 2. Uses of microalgae in different fields (Ferrazzano et al., 2020)

Biochemical Properties of Algae

When applied to soil, microalgae provide a significant source of organic carbon (Alvarez et al., 2021). The decline of soil organic carbon in agricultural lands represents a major form of structural degradation, leading to reduced soil quality and productivity (Stavi and Lal, 2015). In this context, microalgae contribute to carbon sequestration by assimilating atmospheric CO₂ into their biomass through photosynthesis. In addition, extracellular polysaccharides (EPS) secreted by certain strains enhance soil aggregation and structural stability (Alvarez et al., 2021; Costa et al., 2018).

Cyanobacteria and microalgae have been identified as efficient biological agents for the restoration of soils affected by salinity stress (Rocha et al., 2020). These microorganisms are able to produce an extracellular polymeric substance (EPS) layer on the soil surface, which facilitates the retention of organic carbon as well as essential nutrients such as nitrogen and phosphorus. Furthermore,

through the addition of organic matter and nitrogen, they contribute to improved soil aggregation, enhanced permeability, and better aeration conditions (Singh et al., 2016). In addition, EPS produced by these organisms functions as a water-retention matrix under drought stress and helps sustain metabolic activity upon rehydration (Abinandan et al., 2019).

Microalgae, in addition to influencing soil microbial populations, are capable of synthesizing a wide range of bioactive metabolites that promote plant growth and also contribute to the suppression of pests and pathogens (Alvarez et al., 2021). Among the various bioactive compounds produced by microalgae, phenolics, carotenoids, terpenoids, polysaccharides, free fatty acids, and phytohormones have attracted considerable interest because of their important contributions to plant growth stimulation (Gonçalves, 2021).

Microalgal and cyanobacterial biomass contains not only nitrogen and phosphorus but also important macro- and micronutrients essential for plant growth, such as potassium, magnesium, sulfur, and iron. These elements play vital roles in redox reactions and plant metabolic processes (Gonçalves, 2021). In addition, certain cyanobacteria are capable of enhancing the solubility and availability of phosphorus as well as other micronutrients such as zinc, copper, and iron (Rocha et al., 2021; Roychowdhury et al., 2014).

Due to their photosynthetic capacity, algae play an active role in the carbon cycle by converting atmospheric carbon dioxide into biomass. This characteristic makes the use of algae in agriculture not only a yield-enhancing practice but also a supportive strategy in combating climate change (Li and Yao, 2024).

Mechanisms of Microalgae Contribution to Soil Health

Soil health encompasses the diversity of organisms inhabiting the soil, the interactions among these organisms, and the dynamic equilibrium they establish with their environment (Brady and Weil, 2008). Therefore, prioritizing soil health is critically important for ensuring food security, conserving biodiversity, and developing environmentally sustainable agricultural systems (Banerjee and Van Der Heijden, 2023).

The use of microalgae as biofertilizers provides numerous benefits not only for the environment but also for soil health and, consequently, for plant systems. When microalgal biomass comes into direct contact with soil, environmental conditions such as moisture, pH, and light can activate viable microalgal cells, enabling them to maintain metabolic activity and thereby contributing to atmospheric nitrogen fixation (Osorio-Reyes et al., 2023). In addition, cyanobacteria-derived microalgae are considered important due to their capacity for photosynthesis, polysaccharide secretion, and atmospheric nitrogen fixation (Kato et al., 2012).

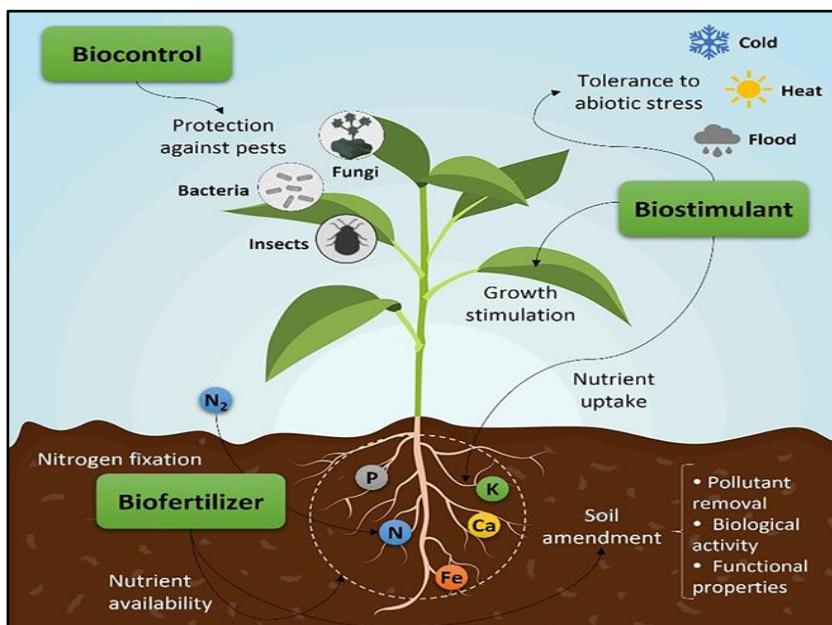


Figure 3. The effects of microalgae-based bioinputs on plant growth and soil health (Ferreira et al., 2023)

Effects on Soil Microbial Activity and Enzyme Functions

Microalgae have a wide range of applications in agricultural production as a potential source of organic carbon (Renuka et al., 2018). The decline in soil organic carbon is one of the key factors leading to reduced soil fertility and, in the long term, the degradation of agricultural lands (Stavi and Lal, 2015). Microalgae convert carbon into sugars through photosynthesis; these sugars influence the structure of soil microbial communities, thereby improving soil aggregation and structural stability (Marks et al., 2019).

In particular, multifunctional microalgae, especially cyanobacteria, contribute to the suppression of pathogens and pests by producing plant hormones and bioactive compounds (Garcia-Gonzalez and Sommerfeld, 2016). In addition, microalgal biomass

represents a resource that can gradually be transformed into nutrients available for plant uptake (Alobwede et al., 2019).

The application of microalgae-derived biostimulants, either alone or in combination with conventional biofertilizers, can enhance nitrogenase enzyme activity in soil (Zhang et al., 2017). These synergistic effects of microalgal biomass on soil and plant systems strengthen the scientific basis for their use in agricultural production systems (Alvarez et al., 2021).

Indeed, various studies have reported that the application of microalgae-based liquid fertilizers at different doses to crops such as cucumber, tomato, pepper, and cowpea significantly increases levels of alkali-hydrolyzable nitrogen, soil organic matter, available phosphorus, and potassium. In addition, increases in soil enzyme activities such as protease and catalase have also been observed to varying degrees (Alobwede et al., 2019).

The effects of microalgae on soil microbial processes are not limited to local nutrient transformations but are also directly linked to global carbon cycling and climate-regulating processes.

Contribution of Microalgae to the Carbon Cycle

Climate change is a global environmental crisis that threatens not only various plant and animal species but also human life. The continuous increase in emissions of greenhouse gases, including carbon dioxide (CO₂), water vapor, methane (CH₄), nitrous oxide (N₂O), and fluorinated gases, is accelerating the process of global warming (Hansen et al., 2000; IPCC, 2014). Atmospheric CO₂ concentration, which was approximately 313 ppm in 1960, has risen to about 411 ppm in recent years (Lindsey, 2020). The increasing atmospheric CO₂ levels also contribute to ocean acidification, significantly affecting marine ecosystems (Joint et al., 2011; Yang et al., 2020). In this context, developing effective strategies to reduce

or stabilize atmospheric CO₂ levels is critically necessary. Although considerable research has been conducted on physical and chemical carbon capture methods, these technologies are limited by high cost and energy requirements. In contrast, biological CO₂ fixation emerges as a more cost-effective and environmentally friendly alternative. Photosynthetic organisms contribute significantly to the regulation of atmospheric carbon dioxide by assimilating CO₂ during the light-independent stages of photosynthesis. Among these organisms, phytoplankton are distinguished by their superior carbon fixation capacity and high biomass production rates. It has been estimated that marine phytoplankton sequester nearly 50 gigatons of CO₂ annually, representing about 50% of the world's primary biological production (Yang et al., 2020).

Research on CO₂ sequestration by microalgae has attracted considerable global attention (Faried et al., 2017; Camerini et al., 2016; Thomas et al., 2016). Compared with vascular plants, microalgae have been reported to assimilate CO₂ at rates 10–50 times more efficiently (Cuellar-Bermudez et al., 2015). Furthermore, it has been estimated in the literature that 1 kg of microalgal biomass can assimilate approximately 1.83 kg of CO₂ (Khan et al., 2018).

These data indicate that microalgae can be considered not only as biofertilizers but also as an integral component of carbon management and climate change mitigation strategies.

Applications of Microalgae in Agriculture

Beyond their essential biological roles in natural ecosystems, microalgae are capable of generating high-value biomass that can be applied as biofertilizers and biostimulants. These microalgae-derived products enhance the biological, chemical, and physical characteristics of soil, while also promoting plant growth and nutrient cycling processes (Osorio-Reyes et al., 2023).

By participating in the decomposition of organic matter, they play an active role in the biochemical cycling of nutrients and influence the availability of these elements for plant uptake in the soil (Balota et al., 1998; Nayak et al., 2019). Furthermore, certain microalgal species have also been reported to contribute to the degradation of toxic compounds (Priya et al., 2014).

The use of microalgal biomass is advantageous not only due to its nitrogen-rich composition but also because of its benefits in carbon sequestration, improvement of soil health, enhancement of soil water-holding capacity, stabilization of soil aggregates, and reduction of nutrient losses (Solé-Bundó et al., 2017).

Use of Microalgae as Biofertilizers

Microalgae are applied in a range of advanced agricultural practices, including foliar spraying, seed coating or priming, and soil amendment strategies. In contrast to chemical fertilizers, the use of microalgae supplies organic carbon to the soil, which helps mitigate the depletion of soil organic carbon and supports the long-term sustainability of soil fertility (Zhang et al., 2024; Renuka et al., 2018). In agricultural use, microalgae are generally applied in the following forms:

(i) live biomass,

(ii) dried biomass, and

(iii) liquid leaf extract formulations (Lu et al., 2022). The application method may vary depending on plant type, growth stage, and the intended effects, such as promoting plant growth, enhancing nutrient uptake, or supporting soil microbial activity. These applications not only contribute to yield improvement and the maintenance of soil health but also help reduce dependence on chemical inputs, mitigate environmental impacts, and strengthen the sustainability of

agricultural systems, thereby supporting climate-smart agricultural approaches (Parmar et al., 2023; Bello et al., 2021).

Accordingly, the positive impacts of microalgae on soil functions and plant growth can be further enhanced and effectively utilized through their use in different formulations such as biofertilizers and biostimulants. Biofertilizers refer to products containing natural compounds and beneficial microorganisms that improve soil characteristics and increase both soil fertility and crop productivity. In contrast, biostimulants are substances or microorganisms that promote plant nutrition by enhancing nutrient use efficiency, stress tolerance, crop quality, and nutrient availability (Miranda et al., 2024).

Use of Microalgae as Biostimulants

Biostimulants are products that, when applied in low doses to seeds, plants, or soil, can regulate and enhance plant physiological processes (Zhang and Schmidt, 1997). These products influence plant physiology through various mechanisms, thereby improving crop growth, yield, quality, nutrient uptake, tolerance to abiotic stresses, and the shelf life of harvested products (Rouphael and Colla, 2018).

Conclusion

The use of biofertilizers in agriculture is increasingly gaining global attention as an alternative or complementary approach to chemical fertilizers, aiming to reduce environmental pollution and soil degradation while maintaining soil fertility and agricultural productivity. Recent advances in microalgae-based biofertilizers present new opportunities for ensuring and improving the sustainable use of this valuable biological resource. These versatile microorganisms enhance soil health and crop productivity while also

making significant contributions to strengthening environmental sustainability.

Microalgae applications can induce positive changes in key soil quality indicators, such as increasing soil organic carbon, enhancing aggregate stability, improving water-holding capacity, and reducing nutrient losses. In particular, cyanobacteria-based applications exhibit structurally regulating effects on soil through biological nitrogen fixation and the production of extracellular polysaccharides, which are of strategic importance for sustainable soil management.

A detailed investigation of the performance of microalgae applications in industrial crops, legumes, and stress-sensitive agricultural systems would further clarify the agronomic value of these biological inputs. Moreover, exploring the synergistic effects of microalgae in combination with organic fertilizers, biogas residues, or other biological inputs may provide new perspectives for the design of sustainable production systems.

In conclusion, microalgae are innovative biological resources capable of simultaneously providing multiple functions, including improving soil health, enhancing plant performance, and supporting carbon management. With appropriate species selection, optimized application strategies, and science-based agronomic optimization, microalgae-based applications are expected to play a significant role in future climate-resilient and sustainable agricultural systems.

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CHAPTER 10

BIOTREMOMOLOGY IN AGRICULTURAL PEST MANAGEMENT: BIOLOGICAL MECHANISMS AND CONTROL STRATEGIES

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1. Introduction

In order to increase agricultural productivity and ensure global food security, the management of pest organisms has become an indispensable component of modern agricultural systems. In this context, classical approaches such as chemical control based on the use of synthetic pesticides and biological control aiming to regulate populations of natural enemies have been widely applied for many years (Bale et al., 2008). In particular, pesticides have often been the primary tool preferred by producers due to their ease of application and short-term economic benefits (Shekhar et al., 2024). However, the intensive and indiscriminate use of chemical control has led to multifaceted crises that threaten the sustainability of agricultural systems. Overreliance on existing pesticides has triggered genetic adaptation in pest populations, resulting in the rapid development of

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pesticide resistance and a consequent decline in the effectiveness of chemical control strategies (Al Nagggar et al., 2025; Satyanarayana, 2024).

In addition to the resistance problem, the dispersion of toxic chemicals within ecosystems disrupts soil microbiota, contaminates water resources, and causes severe adverse effects on non-target organisms, ultimately leading to biodiversity loss (Aksu Altun, 2022; Wan et al., 2025). Furthermore, pesticide residues in agricultural products and environmental exposure increase health risks in humans, including oxidative stress, neurotoxicity, and various chronic diseases (Ergene & Susurluk, 2025; Muñoz-Bautista et al., 2025). These negative outcomes necessitate alternative control methods and integrated approaches that preserve ecological balance, do not harm human health, and prioritize resistance management (Zaffaroni-Caorsi et al., 2022). As a result of this need, biotremology has emerged as a promising biophysical approach in pest management.

Biotremology is an interdisciplinary and rapidly developing field of science that investigates vibrations produced by organisms, propagating as mechanical waves through substrates or across interfaces between environments; their perception via specialized sensory receptors and organs; the dynamic communication processes between sender and receiver; and ultimately their effects on the behavior of living organisms (Cividini & Montesanto, 2020; Gjonov et al., 2025; Hill & Wessel, 2016; Hill & Wessel, 2021; Jocson et al., 2025; Mortimer, 2017; Nieri et al., 2024; Sönmez & Özgen, 2025).

Defined in the literature by Hill and Wessel in 2016, this field was initially considered a sub-discipline of bioacoustics; however, it is clearly distinguished from it in terms of physical transmission medium and sensory perception mechanisms (Nieri et al., 2024). Bioacoustics examines acoustic signals propagating as longitudinal pressure waves in homogeneous fluid media such as gases or liquids

(Cividini & Montesanto, 2020). In contrast, biotremology focuses on mechanical waves propagating through solid substrates (e.g., plant stems, soil) (Gjonov et al., 2025; Mortimer, 2017). Unlike pressure waves, these vibrations propagate along the interface between two media in the form of Rayleigh, Scholte, or Love waves, as well as bending waves (Erbe & Thomas, 2025; Mazzoni et al., 2024).

In bioacoustic analyses, the signals under investigation are detected by auditory receptors capable of sensing pressure fluctuations (Erbe & Thomas, 2025; Hill & Wessel, 2016). In biotremology, however, mechanical waves are perceived not through pressure changes but via specialized mechanoreceptors that detect particle displacement (e.g., subgenual organs in insects and slit sensilla in spiders) (Erbe & Thomas, 2025; Hill & Wessel, 2016; Mazzoni et al., 2024).

2. The Role of Biotremological Approaches in Insect Ecology and Behavioral Sciences

Biotremology refers to a form of communication in which insects utilize mechanically generated vibrations propagating through substrates to perform biological functions such as intraspecific communication, mate selection and mating, territory establishment, and threat detection. It is estimated that more than 200,000 insect species use this mode of communication (Gjonov et al., 2025; Pekas et al., 2024).

2.1. Mating Behaviors

In insects, vibrational communication is most commonly observed in mate location and mating behaviors. This process typically occurs through the reciprocal exchange of species-specific signals (Mazzoni et al., 2024). In many species, communication takes the form of a “duet” between the male calling signal and the female’s response. For example, in *Scaphoideus titanus* (the American grapevine leafhopper), males emit vibrational calling

signals while searching on plants and wait for a response from females; this duet enables the male to locate the female (Cividini & Montesanto, 2020; Zaffaroni-Caorsi et al., 2022).

Similarly, in *Diaphorina citri* (the Asian citrus psyllid), males and females produce synchronized vibrational duets to locate each other (Mankin, 2019; Mankin et al., 2013). Male insects also use vibrational signals from females as a navigation cue. For instance, males of *Nezara viridula* (the southern green stink bug) determine the direction of the female by processing time delays in the arrival of signals reaching their legs (Cividini & Montesanto, 2020).

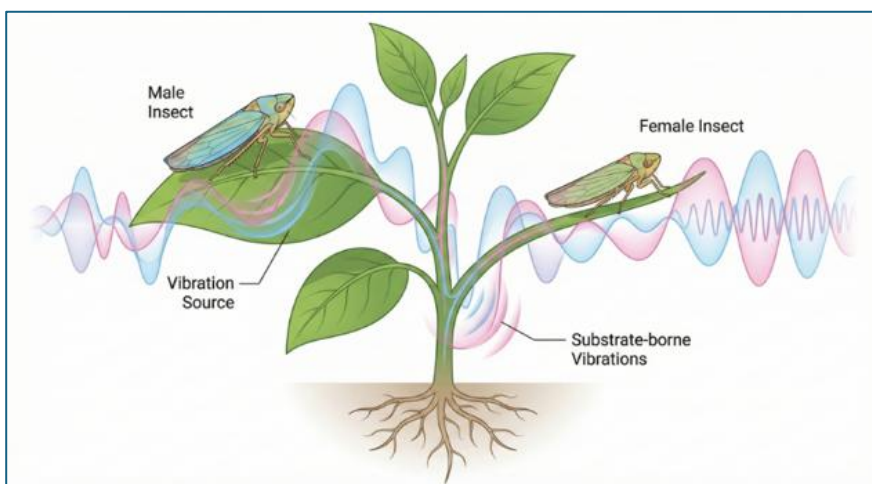


Figure 1: Mechanical wave propagation through substrates in biotremological communication and signal interaction between male and female insects.

2.2. Alarm Signals

In social or group-living insects, vibrations are used to signal the presence of threats to the colony or group. In termite species such as *Zootermopsis angusticollis*, soldiers produce alarm signals during danger by striking their heads against the tunnel ceiling or floor (head-banging) (Erbe & Thomas, 2025). These signals rapidly propagate through the colony, alerting other individuals.

In another example, nymphs of *Umbonia crassicornis* generate synchronized vibrations when a predatory wasp approaches, thereby alerting the mother; the maternal individual responds to these signals, primarily through vibrational and behavioral defenses, to protect the offspring (Cividini & Montesanto, 2020; Mazzoni et al., 2024).

Ants of the genus *Camponotus* produce alarm vibrations by tapping their mandibles and abdomen (gaster) against the substrate (Mazzoni et al., 2024).

2.3. Territoriality

Insects may use vibrational signals to indicate ownership of a territory and to deter rivals. A representative example is males of *Gryllotalpa major* (the great mole cricket), which produce both airborne sound and substrate-borne vibrations via stridulation in their underground calling burrows. These vibrations propagate through the soil and play a regulatory role in mediating competition among neighboring males and in establishing territorial boundaries (Erbe & Thomas, 2025).

2.4. Predator Avoidance

Insects use vibrations both to detect the presence of predators and to avoid being detected by them. In this context, *Aegorhinus nodipennis* (Curculionidae), a hazelnut pest, exhibits a freezing response when it detects vibrations at specific frequencies, remaining motionless. This behavior functions as a defensive mechanism that reduces the likelihood of detection by predators (Stritih-Peljhan et al., 2025).

In addition to defensive strategies, predatory insects can also exploit vibrational cues produced by their prey to locate them. For example, larvae of *Euroleon nostras* (antlion) detect vibrations generated by prey moving across sandy surfaces and initiate attacks

accordingly. Conversely, prey species can also distinguish vibrations generated by predators and develop escape strategies. Indeed, termites such as *Coptotermes acinaciformis* are able to discriminate vibrations produced by walking predatory ants and respond accordingly (Erbe & Thomas, 2025).

2.5. Intraspecific Communication

In social insects, vibrations play a critical role in colony organization and division of labor. In this context, worker honeybees (*Apis mellifera*) produce substrate-borne vibrations on the comb surface through the “tremble dance” and “waggle dance” behaviors to communicate the location of food sources and to regulate nectar foraging activity. In addition, queen bees communicate within the hive via vibrational signals known as “tooting” and “quacking” (Erbe & Thomas, 2025).

The role of vibrational signals is not limited to behavioral coordination; they can also influence morphological development. For example, in wasps of the genus *Polistes*, adults generate vibrations by tapping their antennae on the paper comb of the nest. These vibrations affect larval development and contribute to caste determination (i.e., whether individuals develop into workers or queens) (Mazzoni et al., 2024).

A similar mechanical interaction is observed in leaf-cutting ants (*Atta* spp.), where stridulation during leaf cutting facilitates the cutting process and simultaneously generates short-range substrate-borne vibrational signals that guide other workers toward high-quality food sources (Erbe & Thomas, 2025).

3. Fundamental Principles of Vibrational Communication in Insects

3.1. Mechanisms of Vibrational Signal Production

The mechanisms used by insects to generate vibrational or acoustic signals are generally classified into five main categories: stridulation (friction), percussion (tapping), tremulation (body shaking), tymbal mechanisms (buckling and recovery of tymbal membranes), and sound production via air expulsion (Gjonov et al., 2025; Hill & Wessel, 2016). By employing these mechanisms, insects produce substrate-borne vibrations using a wide variety of body parts.

The primary structures and mechanisms involved in vibrational signal production in insects are as follows:

3.1.1. Stridulation (Friction)

Stridulation is a widely observed acoustic signal production mechanism in arthropods, based on the friction between two specific body structures (Erbe & Thomas, 2025).

Orthoptera (grasshoppers and crickets): Grasshoppers (Caelifera) produce vibrations by rubbing their hind femora against the forewings, whereas crickets (Gryllidae) and katydids (Tettigoniidae) generate sound and vibrations through wing–wing stridulation (Erbe & Thomas, 2025).

Coleoptera: Beetles represent the group with the greatest diversity of stridulatory organs. For example, in some dung beetles (Geotrupidae), stridulation occurs between the hind coxae and the abdomen (coxal–abdominal mechanism), while in longhorn beetles (Cerambycidae), friction occurs between the posterior margin of the pronotum and the mesonotum. Bark beetles (Scolytinae) also produce stridulatory signals using interactions between the elytra and the abdomen (Erbe & Thomas, 2025).

Hymenoptera: Ants (Formicidae) generate vibrations by moving their gaster (abdominal) segments up and down and rubbing the anterior part of the gaster against the post-petiole region. Leaf-cutting ants (*Atta* spp.) also produce stridulatory signals during leaf-cutting activities (Erbe & Thomas, 2025).

Hemiptera (true bugs): Predatory bugs (Reduviidae) produce vibrational signals by rubbing the tip of the rostrum against a grooved area on the prosternum (Erbe & Thomas, 2025).

3.1.2. Percussion (Drumming)

Percussion is a communication mechanism based on insects repeatedly striking a part of their body against a substrate, thereby producing sound and substrate-borne vibrational signals (Erbe & Thomas, 2025; Hill & Wessel, 2016).

Plecoptera (stoneflies): Members of this order generate “drumming” signals by tapping the distal (ventral) part of their abdomen against plant stems or stones (Erbe & Thomas, 2025).

Isoptera (termites): Soldier termites produce alarm signals during danger by striking their heads against the tunnel ceiling or floor (head-banging) (Erbe & Thomas, 2025).

Mantophasmatodea: In this group, males produce vibrations by striking a specialized structure on the subgenital plate against the substrate, while females generate signals by directly tapping their abdomen (Erbe & Thomas, 2025).

Coleoptera: Some species, such as *Xestobium rufovillosum* (the deathwatch beetle), communicate by tapping their heads against tunnel walls (Erbe & Thomas, 2025).

3.1.3. Tremulation (Vibration)

Tremulation is a signal production mechanism in which an insect generates mechanical vibrations—typically transmitted

through the substrate—by rhythmically oscillating part or all of its body without direct tapping on the surface (Erbe & Thomas, 2025; Hill & Wessel, 2016).

Hemiptera: Stink bugs (Pentatomidae), such as *Plautia stali* and *Nezara viridula*, produce signals by vibrating their abdomen up and down, transmitting vibrations through plant tissues (Mazzoni et al., 2017; Yanagisawa et al., 2024). Psyllid species such as *Bactericera cockerelli* also transmit wing and body vibrations to plants via their legs (Avosani et al., 2022).

Diptera (flies): In *Drosophila* species, it has been shown that male courtship signals are actually generated through abdominal tremulation and transmitted as substrate-borne vibrations (Erbe & Thomas, 2025; Hill & Wessel, 2021).

Hymenoptera: Honeybees (*Apis mellifera*) produce vibrational signals such as “stop signals” and communication during the waggle dance by contracting thoracic muscles and vibrating their bodies without wing beating (Erbe & Thomas, 2025).

3.1.4. Tymbal Mechanism (Buckling/Collapse)

The tymbal mechanism refers to the production of clicks and vibrations generated when specialized muscles repeatedly deform (buckling) and release a flexible region of the exoskeleton known as the tymbal.

Hemiptera (Auchenorrhyncha): Cicadas (Cicadidae) produce sound and vibrations by using powerful muscles to buckle the tymbal organs located on the lateral sides of the abdomen (Erbe & Thomas, 2025). Smaller relatives of cicadas, such as leafhoppers (Cicadellidae) and planthoppers (Fulgoromorpha), also possess homologous tymbal organs and use them to generate vibrations that are often inaudible to the human ear and primarily transmitted through substrates (Erbe & Thomas, 2025; Hill & Wessel, 2016).

3.1.5. Air Expulsion

Air expulsion is a communication mechanism in which insects generate acoustic and/or substrate-borne vibrational signals by forcibly expelling air through spiracles or, more rarely, via the mouth.

Blattodea: The Madagascar hissing cockroach (*Gromphadorhina portentosa*) produces both sound and substrate vibrations by expelling air through modified abdominal spiracles (Erbe & Thomas, 2025).

Lepidoptera: Species of the genus *Acherontia* (death's-head hawkmoths) generate sound and vibrations by inflating and deflating the pharynx; this action causes a lobe in the epipharyngeal region to vibrate (Erbe & Thomas, 2025).

3.2. Detection of Substrate Vibrations

The perception of substrate-borne vibrations in insects is generally mediated by mechanoreceptors. Two main groups of sensory organs are involved in the detection of vibrational and acoustic signals in insects. One group is always associated with the exocuticle, while the other consists of internally located receptor clusters connected to the integument (Erbe & Thomas, 2025).

3.2.1. Subgenual Organ (SGO)

The subgenual organ (SGO) has been observed in almost all insect orders except Diptera (flies) and Coleoptera (beetles), and is located in the tibial segment of the legs (Erbe & Thomas, 2025; Hill & Wessel, 2021). SGOs function as proprioceptors and also serve as the primary organs responsible for the perception of vibrations and/or sound (Erbe & Thomas, 2025).

3.2.2. Chordotonal Organs

In species lacking a subgenual organ, such as *Drosophila* and some Coleoptera groups (e.g., Cerambycidae), substrate-borne

vibration detection is performed by the femoral chordotonal organ (FCO) located in the legs (Erbe & Thomas, 2025; Hill & Wessel, 2021; Stritih-Peljhan et al., 2025). In contrast, the Johnston's organ, located in the second antennal segment (pedicel), plays a key role in detecting near-field sound by sensing antennal movements, particularly in mosquitoes and *Drosophila* (Erbe & Thomas, 2025; Hill & Wessel, 2021).

3.2.3. Cuticular Sensilla (External Receptors)

Various types of sensilla located on the body surface also contribute to vibration perception:

Trichoid sensilla: These are hair-like structures (setae) attached to the cuticle via a flexible membrane. When external mechanical stimuli bend the hair, the associated neuron is activated. These structures are sensitive to touch and vibrations. For example, in antlions (*Euroleon nostras*), bundles of setae called “sensilla chaetica” on the thorax play an important role in detecting prey-generated vibrations transmitted through sand (Erbe & Thomas, 2025).

Campaniform sensilla: These are dome-shaped structures located in cuticular depressions. They detect deformation and mechanical stress in the cuticle, thereby providing vibration-related sensory information (Cividini & Montesanto, 2020; Erbe & Thomas, 2025).

Campaniform-type mechanoreceptors: These structures consist of a cuticular dome that senses deformation of the exoskeleton. They respond to mechanical strain and deformation of the body wall. In crickets, they are found on the cerci and near leg joints, while in Diptera and Strepsiptera they are often located at the wing bases (Cividini & Montesanto, 2020; Erbe & Thomas, 2025).

Trichobothria / filiform mechanoreceptors: These hair-like receptors are involved in detecting weak air currents, low-frequency sounds, and vibrations. They consist of a hair attached to the cuticle

via elastic fibers and a membrane, where deflections of the hair tip are transmitted to sensory dendrites. Well characterized in crickets, these receptors contribute to predator detection (e.g., approaching predators from behind) and the initiation of escape responses. In spiders, they are referred to as trichobothria and function in prey detection. Across arthropods, they are located on various body parts such as cerci, antennae, body segments, legs, and pedipalps (Cividini & Montesanto, 2020).

4. Insect Control Through Biotremology

4.1. Vibrational Mating Disruption (VMD): Masking and Noise-Generation Strategies

Vibrational Mating Disruption (VMD) is a biotechnical method based on preventing pre-mating communication between individuals through species-specific artificial vibrations, with the aim of minimizing the reproductive success of target pests. This strategy operates by deploying artificial signals in agricultural environments (e.g., vineyards, greenhouses), typically defined as disturbance noise. The core mechanism of the system is masking, in which a receiver (e.g., a male insect) is unable to detect or discriminate the signal from the sender (female individual) due to dominant artificial noise in the environment (Figure 2).

During the masking process, artificial noise or signals covering the communication frequency range of the pest species are introduced into the environment. This noise overpowers or masks the natural signals, thereby disrupting the natural duetting process between male and female individuals (Mazzoni et al., 2017; Zaffaroni-Caorsi et al., 2022). As a result, the male is unable to locate the female, leading to inhibition or delay of mating. This ultimately contributes to the suppression of pest populations.

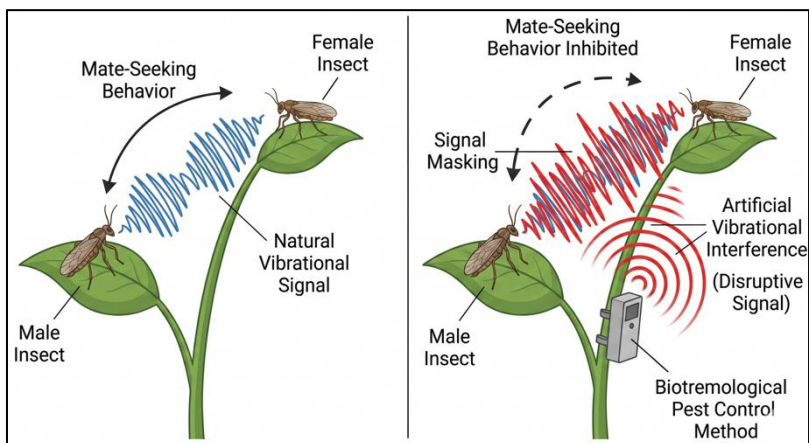


Figure 2: Mechanism of Vibrational Mating Disruption (VMD) in biotremological pest management, showing the masking of natural substrate-borne signals by artificial vibrations and the resulting inhibition of mating.

For example, in *Euschistus heros*, when noise overlapping the communication frequency is applied, it has been observed that even though females continue to emit calling signals, males are unable to process the signals and consequently fail to locate the female (Avosani et al., 2022). The masking signals used for this purpose can be delivered either as a continuous stream or in specific rhythmic patterns designed to prevent insects from exploiting signal gaps.

The VMD method and its associated masking mechanism have been shown to be effective against the following species, whose communication largely depends on vibrational signals:

- *Scaphoideus titanus* (American grapevine leafhopper): (Nieri et al., 2023; Zaffaroni-Caorsi et al., 2022)
- *Hebata (Empoasca) vitis* (leafhopper): (Nieri et al., 2023)
- *Homalodisca vitripennis* (glassy-winged sharpshooter): (Avosani et al., 2022)
- *Diaphorina citri* (Asian citrus psyllid): (Mankin, 2019)

- *Bemisia tabaci* and *Trialeurodes vaporariorum* (whiteflies): (Yanagisawa et al., 2024)
- *Neoempheria* sp. (fungus gnats): (Yanagisawa et al., 2024)

4.2. Vibrational-Based Trapping Using Deceptive Signals

The use of vibrational signals in agricultural pest management encompasses a range of strategies aimed at population control by manipulating insect behavior. These strategies can be broadly grouped into three main categories.

4.2.1. Competitive Mating Disruption

In this method, a synthetic copy (mimic) of the female response signal of the target species is broadcast, with the aim of diverting males toward false females instead of real ones (Jocson et al., 2025). As males are attracted to the artificial source, mating success is reduced.

4.2.2. Luring/Trapping and “Attract-and-Kill” Strategy

This approach involves mimicking natural vibrational signals used in intraspecific communication (e.g., calling or aggregation signals) to attract individuals to a specific location (vibrational trapping/luring). Once insects are drawn to the synthetic signal source, they are eliminated using physical (e.g., adhesive surfaces) or chemical (e.g., insecticides) control mechanisms (Mankin et al., 2013; Vaughn et al., 2024).

4.2.3. Loitering Effect

The loitering effect refers to the prolonged residence of a male individual near an artificial signal source after perceiving it as a receptive female. This behavior spatially and temporally disrupts the male’s ability to locate real females, thereby reducing reproductive success (Mazzoni et al., 2017).

4.3. Biotremological Pest Management in Different Ecosystems and Application Examples

Biotremology-based strategies have been successfully tested and implemented under field conditions across different ecosystems and target pest species.

One of the most extensive agricultural applications of biotremology has been conducted on *Scaphoideus titanus*, the vector of Flavescence dorée phytoplasma. In multi-year studies (over five years) carried out in the Trentino region of Italy, disturbance noise (DN) was transmitted into plant tissues via electromagnetic transducers (shakers) integrated into vineyard trellis support wires. Through masking signals that disrupted species-specific mating duets, reductions of 36% to 63% were recorded in *S. titanus* nymph populations (Nieri et al., 2023). Further optimization of the system, including the addition of 250 Hz pure tones to the DN signal, also suppressed populations of the leafhopper *Hebata vitis* by 30%–53%. These findings demonstrate that multiple taxonomic groups can be controlled simultaneously using a single physical infrastructure.

In the management of *Halyomorpha halys*, a bimodal approach combining chemical and physical stimuli has introduced a new paradigm in monitoring and mass trapping. A major limitation of commercial pheromone traps—trap spillover, where insects aggregate near but do not enter traps—has been overcome through vibrational cues. Vibrotrap systems combining the female “Female Song 2” (FS2) signal with pheromones have captured both male and female individuals at significantly higher rates (Zapponi et al., 2023). In this system, pheromones function as long-range attractants, while the FS2 signal acts as a short-range localization cue that guides insects into the trap (Mazzoni et al., 2017).

In studies targeting the spread of Huanglongbing (HLB), vibrational duet mechanisms of *Diaphorina citri* have been

manipulated using AI-supported systems. Devices capable of detecting male calls in real time and emitting synthetic female response signals within microseconds disrupted natural mate-searching behavior. As a result, mating success in citrus orchards decreased from 57% to 13%, with approximately half of searching males being diverted toward artificial signal sources (Mankin, 2019).

In greenhouse systems, studies targeting whiteflies (*Bemisia tabaci* and *Trialeurodes vaporariorum*) in tomato (*Solanum lycopersicum*) production have shown that vibrations transmitted through support wires in the 100–300 Hz range suppress nymph populations by 40%–50%. A similar approach applied in shiitake mushroom cultivation against *Neoempheria* spp. delayed larval pupation through intermittent vibration exposure. Additionally, low-frequency vibrations (~30 Hz) have been reported to enhance pollination efficiency in tomato plants and increase fruit set by stimulating pollinator activity (Yanagisawa et al., 2024).

Biotremology-based applications are increasingly integrated as complementary components of Integrated Pest Management (IPM) strategies rather than standalone solutions. A clear example of this integration is bimodal trapping systems that combine chemical cues (semiochemicals) with physical signals (semiphysical cues) (Mazzoni et al., 2024). In studies on the brown marmorated stink bug (*Halyomorpha halys*), the effectiveness of commercial pheromone traps was significantly enhanced when supplemented with species-specific vibrational signals (Zapponi et al., 2023). In these systems, pheromones attract insects from long distances, while vibrations promote trap entry at short range, reducing trap spillover effects.

The integration of biotremology with biological control and cultural practices is also considered within the framework of the ecology of fear. Mimicking predator footsteps or vibrations can induce avoidance behavior in pests, while predators can be pre-conditioned to vibrational cues of their prey to enhance hunting

efficiency (Pekas et al., 2024). However, since some parasitoid wasps also rely on vibrational cues to locate their hosts, careful timing of artificial vibration deployment is necessary to avoid negative impacts on beneficial insects (Yanagisawa et al., 2024). Furthermore, certain vibration frequencies used for whitefly control in greenhouses (e.g., 100–300 Hz) have been reported to enhance bumblebee pollination efficiency or provide additional mechanical pollination benefits in tomato crops (Yanagisawa et al., 2024).

5. Conclusion

Biotremology offers a novel perspective in modern pest management by focusing on substrate-borne vibrations used by insects for communication and environmental perception, through semiphysical tools. Positioned at the intersection of biology, ecology, and engineering, this approach enables highly precise manipulation of insect behavior through methods such as vibrational mating disruption, bimodal trapping, and predator signal-based deterrence. Unlike chemical pesticides, its lack of toxic residues on agricultural products and its species-specific nature, which helps protect non-target organisms, make it a strategic solution for organic farming systems and strict food safety standards.

Being highly compatible with Integrated Pest Management (IPM) systems and relying on physical mechanisms that reduce the likelihood of resistance development in pests, this discipline supports sustainable production models aimed at minimizing agricultural inputs. Owing to these characteristics, biotremology provides an environmentally compatible and durable plant protection strategy that overcomes the ecological and biological limitations of conventional chemical control.

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CHAPTER 11

THE HIDDEN HEROES OF THE NITROGEN CYCLE: COMAMMOX BACTERIA

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Introduction

Complete ammonia-oxidizing (comammox) bacteria are a recently discovered group of microorganisms within the phylum *Nitrospirota* that possess the unique ability to convert ammonia (NH₃) directly to nitrate (NO₃⁻) through a single metabolic pathway (Daims et al., 2015; van Kessel et al., 2015). Owing to this capability, they can independently carry out the complete nitrification process, which was previously believed to require the coordinated activity of

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distinct microbial groups responsible for ammonia oxidation and nitrite oxidation, respectively. The discovery of comammox bacteria has significantly advanced our understanding of the nitrogen cycle, particularly in agricultural soils and wastewater treatment systems.

These microorganisms have been shown to play an important role in enhancing soil fertility and improving nitrogen use efficiency in crops such as maize (Kits et al., 2017; Wang et al., 2020). Nevertheless, the environmental distribution, ecological functions, and interactions of comammox bacteria with other nitrifying microorganisms remain incompletely understood. In particular, elucidating their responses to different fertilization practices in agricultural soils is crucial for the development of sustainable nitrogen management strategies. Therefore, further research on comammox bacteria is expected to contribute not only to a deeper understanding of soil microbiology but also to the mitigation of environmental nitrogen losses.

The ecological significance of comammox bacteria is closely linked to their interactions within complex microbial communities and their sensitivity to environmental factors such as soil pH, moisture content, oxygen availability, and temperature (Hu & He, 2017). Studies have demonstrated that these bacteria can thrive under specific environmental conditions and establish both competitive and cooperative relationships with ammonia-oxidizing archaea, ammonia-oxidizing bacteria, and nitrite-oxidizing microorganisms. These interactions can substantially influence nitrification rates, nitrogen retention in soils, and the emission of greenhouse gases such as nitrous oxide (N₂O) (Kuypers et al., 2018).

Consequently, comammox bacteria are regarded not only as an independent functional group involved in the regulation of the nitrogen cycle but also as a key component shaping the structure and functioning of microbial networks. Furthermore, their sensitivity to

environmental changes highlights the need for a deeper understanding of how climate change and soil management practices influence these microbial communities.

Recent research has also revealed that comammox bacteria possess remarkable metabolic versatility. In addition to carrying out complete nitrification, certain species have been found to utilize alternative energy sources and possess carbon fixation pathways that enable their survival in nutrient-limited or acidic environments (Palomo et al., 2022). These physiological characteristics enhance their ecological adaptability across diverse habitats. Nevertheless, the environmental distribution, abundance, and community dynamics of comammox bacteria remain insufficiently understood, which currently limits their effective application in agricultural and engineering contexts (Pjevac et al., 2017). Therefore, future studies should focus on elucidating their functional roles in different ecosystems and their responses to environmental changes in greater detail.

Comammox bacteria, which possess the ability to oxidize ammonia, are chemoautotrophic microorganisms belonging to the phylum *Nitrospirota*, formerly referred to as *Nitrospirae*. These bacteria are distinguished from other nitrifying microorganisms by their capacity to perform both steps of the nitrification process—the oxidation of ammonia to nitrite and the subsequent oxidation of nitrite to nitrate—within a single cell. While the conventional model of nitrification assumes that these two reactions are carried out by distinct groups of microorganisms, the discovery of comammox bacteria has prompted a reassessment of existing knowledge regarding the nitrogen cycle (Liu et al., 2022). This finding has enabled a more integrated understanding of nitrification at the microbial level and has contributed to the reinterpretation of nitrogen transformation dynamics, particularly in soil and aquatic ecosystems.

2. Metabolism and Biochemistry of Comammox Bacteria

Comammox bacteria are among the recently identified groups of nitrifying microorganisms and possess unique metabolic characteristics that contribute significantly to the nitrogen cycle in terrestrial ecosystems. Their most remarkable feature is their ability to perform complete nitrification. In other words, they can oxidize ammonia (NH_3) directly to nitrate (NO_3^-), thereby completing within a single organism a process that was traditionally thought to require two distinct groups of microorganisms (Hu & He, 2017). Through this capability, comammox bacteria contribute to more efficient nitrogen transformation processes, particularly in nutrient-poor soils, and support the formation of nitrogen compounds that are readily available for plant uptake in ecosystems.

The metabolism of comammox bacteria is primarily based on the activity of key enzymes, including ammonia monooxygenase (AMO) and hydroxylamine dehydrogenase (HAO). In the first step, ammonia is converted to hydroxylamine by the AMO enzyme, which is subsequently oxidized to nitrite through the action of HAO. In the final stage, the enzyme nitrite oxidoreductase (NXR) catalyzes the oxidation of nitrite to nitrate. This biochemical pathway is closely coupled to the electron transport chain for energy generation, thereby fulfilling the cellular energy requirements of the organism (Kits et al., 2017). Moreover, this process supports the chemolithoautotrophic lifestyle of comammox bacteria by enabling the energy derived from inorganic nitrogen compounds to be utilized for carbon fixation and cellular growth.

Comammox bacteria are chemolithoautotrophic organisms that derive their energy primarily from the oxidation of inorganic nitrogen compounds. In addition, they are capable of converting carbon dioxide into biomass through carbon fixation mechanisms such as the Calvin–Benson–Bassham cycle (Palomo et al., 2018).

The ability of certain species to utilize alternative nitrogen sources, including urea, further enhances their metabolic flexibility and enables them to persist under a wide range of environmental conditions (Koch et al., 2019). This metabolic versatility provides comammox bacteria with a competitive advantage, particularly in nutrient-limited environments, and further underscores their critical role in the nitrogen cycle.

From a biochemical perspective, the high affinity of comammox bacteria for low ammonium concentrations provides them with a competitive advantage in oligotrophic, nutrient-limited environments. This characteristic helps explain their widespread occurrence in agricultural soils, biofilm-based systems, and groundwater habitats (Fowler et al., 2018). Furthermore, several studies have suggested that comammox bacteria may produce lower levels of nitrous oxide (N₂O) than conventional ammonia-oxidizing bacteria, a feature that is particularly relevant from the standpoint of environmental sustainability (Hu & He, 2017). Consequently, comammox bacteria are regarded as an important microbial group for sustainable ecosystem management due to their potential to enhance the efficiency of the nitrogen cycle while simultaneously contributing to the reduction of greenhouse gas emissions.

2.1. Nitrogen Metabolism

The metabolic characteristics of comammox *Nitrospira* species have been elucidated in considerable detail through genomic analyses. Studies have demonstrated that these bacteria possess a wide range of key genes involved in nitrogen metabolism. In particular, the presence of genes encoding nitrate reductase and nitrite reductase enzymes indicates that comammox bacteria may participate not only in nitrification but also in nitrate reduction and nitrogen assimilation processes (Zhao et al., 2025). These findings suggest that comammox bacteria possess a more flexible and

versatile metabolic capacity than previously recognized, thereby helping to explain their ability to adapt to diverse environmental conditions and their multifaceted role in the nitrogen cycle.

In comammox bacteria, the *nirBD* genes encode the cytoplasmic nitrite reductase enzyme, which catalyzes the reduction of nitrite to ammonia. This process is considered an important assimilatory mechanism, particularly for meeting cellular nitrogen requirements. Similarly, the *narGHI* gene cluster encodes the membrane-bound nitrate reductase complex responsible for the reduction of nitrate to nitrite. These genetic features demonstrate the metabolic adaptability of comammox bacteria to changing environmental conditions (Koch et al., 2019). They further indicate that these microorganisms are actively involved not only in the nitrification process but also in pathways associated with the cellular utilization of nitrogen, thereby enabling them to adopt a more flexible metabolic strategy within the nitrogen cycle.

One of the most significant features of comammox bacteria in nitrogen metabolism is their ability to perform complete nitrification. In this process, ammonia is first oxidized to nitrite and subsequently to nitrate. Unlike the conventional nitrification model, these two reactions are carried out by a single organism. Furthermore, genomic analyses have revealed that some comammox species possess a urea metabolism pathway. Through the activity of the urease enzyme system, urea is hydrolyzed to release ammonia, which can then be utilized as an energy source during the nitrification process (Daims et al., 2015). This metabolic capability enables comammox bacteria to exploit alternative nitrogen sources, particularly in environments characterized by low ammonium concentrations, thereby enhancing their ecological competitiveness and adaptive success.

This metabolic flexibility provides comammox bacteria with a significant ecological advantage in nutrient-poor environments. Their ability to utilize a variety of nitrogen compounds, particularly in agricultural soils, biofilm systems, and groundwater habitats, enhances their functional importance within the nitrogen cycle. Furthermore, these bacteria have been reported to carry out nitrogen transformation processes more efficiently and, in some cases, to produce lower levels of N₂O emissions, a characteristic of considerable relevance to environmental sustainability (Hu & He, 2017; Fowler et al., 2018). Owing to these attributes, comammox bacteria are increasingly recognized as an important microbial group with substantial potential to contribute to the optimization of nitrogen management in both natural ecosystems and engineered systems.

2.2. Carbon Fixation

Comammox bacteria are microorganisms that play important roles not only in nitrogen metabolism but also in the carbon cycle. Genomic and metabolic studies have demonstrated that these bacteria primarily utilize the reverse tricarboxylic acid (rTCA) cycle for carbon fixation (Zhao et al., 2025). Through this metabolic pathway, comammox bacteria are able to convert atmospheric or dissolved carbon dioxide (CO₂) into organic carbon compounds under specific environmental conditions.

The rTCA cycle is known as an energy-efficient carbon fixation mechanism and is commonly found in chemolithoautotrophic microorganisms adapted to low-energy environments. The use of this pathway by comammox bacteria contributes to their ability to survive in oligotrophic, nutrient-poor conditions (Kits et al., 2017). In addition, the energy generated during nitrification is utilized for the conversion of CO₂ into biomass. This indicates that comammox bacteria are capable of

integrating carbon and nitrogen cycles in a coordinated manner, thereby making a significant contribution to both energy flow and nutrient cycling within ecosystems.

The capacity for carbon fixation suggests that comammox bacteria may influence not only the nitrogen cycle but also soil carbon dynamics. In particular, it is thought that these microorganisms can simultaneously affect carbon and nitrogen cycling in agricultural soils and biofilm systems (Lawson & L  cker, 2018). This dual functionality enhances their ecological significance and makes them particularly relevant in the context of sustainable agricultural practices. Consequently, comammox bacteria are considered a critical microbial group for the maintenance of soil fertility and the long-term stability of carbon–nitrogen balance in ecosystems.

2.3. Adaptation to Environmental Conditions

Comammox bacteria possess a range of genetic and physiological adaptation mechanisms that enable their survival under diverse environmental conditions. Their ability to tolerate acidic environments is a key trait explaining their widespread occurrence in agricultural soils, biofilm systems, and low-oxygen habitats. Genomic analyses have revealed that comammox bacteria harbor genes encoding the *kdpABC* potassium transport system. This system helps maintain the electrochemical balance across the cell membrane and supports the preservation of an inverted membrane potential under acidophilic conditions (Palomo et al., 2018; Lawson & L  cker, 2018). Such adaptive mechanisms facilitate the maintenance of metabolic activity under environmental stress and provide these organisms with a competitive advantage across different ecosystems.

The potassium transport system plays an important role in maintaining intracellular pH stability and ensuring the continuity of

cellular metabolism under low-pH conditions. Through this adaptation, comammox bacteria are able to thrive in mildly acidic soils and environments with low dissolved oxygen (DO) levels. It has been reported that under low-oxygen conditions, they tend to favor shorter and more energy-efficient metabolic pathways in order to enhance energy efficiency (Kits et al., 2017). These characteristics enable comammox bacteria to sustain nitrification processes even under environmental stress, and they are considered one of the key factors explaining their ecological success across diverse habitats.

In addition, comammox bacteria possess enzyme systems with high affinity for substrates at low concentrations. This characteristic provides them with a competitive advantage over other nitrifying microorganisms in oligotrophic, nutrient-poor environments. Studies have shown that comammox species are capable of maintaining active nitrification even at low ammonium levels and have developed metabolic strategies that optimize energy utilization (Fowler et al., 2018). As a result, comammox bacteria are able to sustain nitrification processes even in ecosystems where nitrogen is limited, thereby making a significant contribution to the continuity of the nitrogen cycle.

These environmental adaptations indicate that comammox bacteria may play an important role not only in natural ecosystems but also in engineered applications such as wastewater treatment systems. In particular, their low oxygen requirements and high energy efficiency are considered notable characteristics for sustainable nitrification processes (Shi et al., 2018; Koch et al., 2019). Owing to these features, they are thought to have the potential to contribute to more stable and efficient nitrogen removal in treatment systems.

3. Alternative Energy Metabolisms

Comammox bacteria are not limited to classical nitrification pathways but are metabolically flexible microorganisms capable of utilizing diverse energy sources. Genomic studies have revealed that these bacteria possess genetic potential for various alternative energy metabolisms, including hydrogen oxidation, cyanate utilization, and guanidine metabolism. These features contribute to their ability to persist under different environmental conditions and enhance their overall energy efficiency (Yang et al., 2020; Xu et al., 2020). This metabolic diversity enables comammox bacteria to gain a competitive advantage, particularly in nutrient-poor and environmentally variable ecosystems, further emphasizing their important role in the nitrogen cycle.

In particular, some comammox species have been shown to utilize hydrogen as an electron donor. Through hydrogen oxidation, an additional input of energy is provided to the electron transport chain, thereby supporting the continuation of metabolic activity under low-nutrient conditions. This metabolic strategy is considered an important adaptive mechanism in energy-limited environments (Wu et al., 2024; Zhang et al., 2025). It contributes to the ability of comammox bacteria to maintain energy balance even under environmental stress, thereby enhancing their ecological success across diverse ecosystems.

Another notable feature of comammox bacteria is their ability to utilize unconventional nitrogen compounds such as cyanate (OCN^-). Cyanate can be decomposed into ammonium and carbon dioxide via the action of the enzyme cyanase, and the released ammonium can subsequently be used as an energy source in the nitrification process. This mechanism provides a significant ecological advantage, particularly in environments where organic nitrogen turnover is high (Li et al., 2023; Xiang et al., 2025). In this way, comammox bacteria are able to exploit different chemical

forms of nitrogen, thereby contributing to the continuity and efficiency of nitrogen cycling in ecosystems.

In addition, some comammox species have been reported to be capable of guanidine metabolism, whereby guanidine is degraded to produce ammonium. This transformation provides an additional source of nitrogen and energy, thereby increasing their metabolic diversity. Such a capability offers a survival advantage, particularly in environments with low ammonium concentrations (Guo et al., 2026). It further demonstrates the ability of comammox bacteria to utilize a wide range of nitrogen-containing organic compounds, strengthening their adaptability to variable environmental conditions.

Through their alternative energy metabolisms, comammox bacteria are able to achieve higher energy efficiency compared to conventional nitrifying microorganisms and, in some cases, produce lower levels of nitrous oxide (N_2O). For this reason, they are often regarded as “green nitrifiers.” In particular, their potential to reduce greenhouse gas emissions during the efficient removal of nitrogen compounds in wastewater treatment systems is of great importance for sustainable environmental technologies (Deogratias et al., 2025; Zu et al., 2025). Overall, these characteristics suggest that comammox bacteria may contribute to the optimization of nitrogen transformation processes with a reduced environmental footprint in both natural ecosystems and engineered systems.

3.1. Effects of Environmental Factors

The distribution, activity, and ecological functions of comammox bacteria are strongly influenced by environmental conditions. In particular, factors such as soil pH, moisture content, temperature, and dissolved oxygen levels are among the key environmental variables that determine the growth and nitrification capacity of these microorganisms (Zhao et al., 2025). These factors

shape not only the physiology of comammox bacteria but also their interactions with other microbial communities.

Soil pH is considered one of the most important factors influencing the environmental distribution of comammox bacteria. Low pH conditions can directly affect nitrification processes by altering the structure of microbial communities. Studies have shown that certain comammox species exhibit high tolerance to mildly acidic conditions and may gain a competitive advantage over ammonia-oxidizing bacteria in such environments (Sun et al., 2021; Chisholm et al., 2024). This allows comammox bacteria to occupy a broad ecological niche across different soil types and suggests that they may play an important role in sustaining nitrification processes, particularly in acidic agricultural soils.

Under environmental stress conditions, interactions among microbial communities may also change. In particular, under abiotic stress factors such as low pH, limited oxygen availability, and nutrient scarcity, competitive relationships may be replaced by more cooperative interactions. This phenomenon is explained by the “stress gradient hypothesis,” which proposes that under harsh environmental conditions, symbiotic or mutually beneficial relationships among microorganisms may develop (Qi et al., 2018). In this context, it is suggested that the interactions of comammox bacteria with other nitrifying microorganisms may be reshaped depending on environmental conditions, thereby contributing to a more balanced regulation of nitrogen transformation processes at the ecosystem level.

These interactions between comammox bacteria and other nitrifying microorganisms can enhance the efficiency of nitrogen transformation processes. In particular, within biofilm structures or complex soil microbiomes, metabolic exchange of intermediates among different microbial groups may occur, thereby contributing to

the continuity of nutrient cycling. In this way, comammox bacteria play an important role in ecosystem processes not only through their individual metabolic activities but also through their functional interactions within microbial communities (Palomo et al., 2018). These interactions promote more stable and efficient nitrogen turnover, contributing to the maintenance of overall biogeochemical balance in ecosystems.

Soil moisture and temperature also significantly influence the activity of comammox bacteria. Changes in moisture levels affect oxygen diffusion and substrate transport, whereas temperature determines the rate of enzymatic reactions. It has been reported that nitrification performance of comammox bacteria increases under moderate temperature and adequate moisture conditions (Kits et al., 2017). However, extreme stress conditions such as severe drought or high temperatures may lead to a reduction in microbial activity.

These environmental sensitivities indicate that the role of comammox bacteria in ecosystem functioning may vary considerably depending on changing environmental conditions. In particular, factors such as climate change, soil acidification, and agricultural practices are thought to have significant impacts on the structure of comammox communities and on nitrification processes.

3.2. Nitrification and Its Agricultural Impacts

The activity of comammox bacteria is of critical importance for agricultural productivity, particularly in relation to soil nitrogen cycling. The application of both organic and inorganic fertilizers can significantly enhance the potential nitrification activity (PNA) of soils, thereby influencing maize productivity and nitrogen use efficiency (NUE). Research has shown that appropriate fertilization strategies can optimize microbial activity, which in turn increases crop yield while reducing nitrogen losses through emissions of nitrous oxide (N₂O), a potent greenhouse gas (Yang et al., 2024).

Therefore, considering comammox bacteria in conjunction with fertilization management represents an important approach for developing sustainable agricultural practices aimed at improving productivity while minimizing environmental impacts.

Organic and inorganic fertilizers used in agricultural practices can significantly increase the potential nitrification activity (PNA) in soils. Ammonium supplied through fertilization serves as a primary substrate for comammox bacteria, thereby stimulating microbial nitrification processes. Studies have shown that balanced fertilization strategies not only improve plant nutrition but also enhance nitrogen use efficiency (NUE) by optimizing microbial activity (Shi et al., 2018). These findings indicate that fertilizer management should be designed not only with a focus on productivity but also by considering microbial ecology, which is essential for sustainable agriculture.

In particular, organic fertilizer applications are reported to support the development of comammox communities by increasing soil organic matter. The slow-release nitrogen sources resulting from the decomposition of organic materials provide suitable conditions for comammox bacteria, which are adapted to low substrate concentrations. In contrast, excessive use of chemical fertilizers may lead to soil acidification and disturbances in microbial balance, thereby negatively affecting nitrification processes (Hu & He, 2017). Therefore, adopting a balanced approach between organic and inorganic fertilizer inputs is important both for maintaining the activity of comammox bacteria and for ensuring long-term soil health and sustainability.

One of the most important agricultural advantages of comammox bacteria is their potential to reduce nitrogen losses. In conventional nitrification processes, significant amounts of nitrous oxide (N_2O) can be produced, and this gas contributes to

environmental problems as a potent greenhouse gas. Some studies suggest that comammox bacteria may lead to lower N₂O emissions due to their more efficient energy metabolism (Kits et al., 2017). For this reason, comammox bacteria are attracting increasing attention in the context of sustainable agriculture and climate-friendly nitrogen management strategies.

In addition, appropriate fertilization practices that support the activity of comammox bacteria can accelerate nitrogen transformation processes in the rhizosphere, thereby enhancing crop productivity. In particular, practices such as controlled fertilization, the use of organic amendments, and maintaining optimal soil pH can strengthen the functional role of these bacteria in agricultural ecosystems (Fowler et al., 2018). Considering microbial processes in soil management can make an important contribution to sustainable agricultural practices by improving nutrient use efficiency and reducing environmental nitrogen losses.

4. Role of Comammox Bacteria in the Nitrogen Cycle

Comammox (complete ammonia-oxidizing) bacteria play a vital role in the nitrogen cycle by facilitating the transformation between ammonia (NH₃) and nitrate (NO₃⁻) in agricultural soils. These microorganisms contribute significantly to the regulation of nitrogen fixation processes and nitrous oxide (N₂O) emissions, both of which are critical for maintaining soil health and crop productivity (Fudjoe et al., 2023). In this way, comammox bacteria enhance the efficient utilization of nitrogen in agricultural ecosystems, thereby increasing crop yield while reducing environmental nitrogen losses.

These bacteria contribute to crop development, particularly in agricultural ecosystems, by facilitating the conversion of nitrogen into forms that can be readily taken up by plants. The nitrate produced through nitrification is one of the primary nitrogen sources for plants and has a significant impact on growth and yield,

especially in high nitrogen-demand crops such as maize. Therefore, the presence of comammox bacteria can enhance nitrogen use efficiency (NUE), thereby positively influencing both plant productivity and soil health (Xu et al., 2020; Wang et al., 2020). When considered in conjunction with optimized fertilization management, this contributes to economically efficient agricultural production as well as improved environmental sustainability.

5. Effects of Fertilization Practices

Fertilization practices significantly influence the composition and functional diversity of microbial communities, including comammox bacteria. Studies have shown that both organic and inorganic fertilizers can increase the activity and abundance of ammonia-oxidizing bacteria (AOB) and comammox bacteria (CAOB) under different soil nitrogen conditions (Liu et al., 2022; Fudjoe et al., 2023). In particular, the addition of organic fertilizers has been shown to improve soil properties by increasing organic carbon levels, which in turn enhances nitrification rates and reduces N₂O emissions (Fudjoe et al., 2023). These relationships highlight the need for fertilization strategies that consider microbial community dynamics in order to optimize agricultural productivity while minimizing environmental impacts.

The responses of comammox bacteria to fertilization practices highlight the importance of soil management strategies for sustainable agriculture. In particular, organic matter-rich fertilization practices can support microbial activity and contribute to more balanced nitrogen transformation processes. This is considered important both for improving plant nutrient use efficiency and for reducing environmental nitrogen losses.

6. Conclusions

In conclusion, comammox bacteria represent a functionally important and ecologically versatile group within the nitrogen cycle. Their ability to perform complete nitrification, combined with metabolic flexibility and adaptation to diverse environmental conditions, distinguishes them from other nitrifying microorganisms. Evidence indicates that they contribute to improved nitrogen use efficiency, enhanced soil fertility, and potentially reduced nitrous oxide emissions. Their activity is strongly influenced by environmental factors and fertilization practices, highlighting their relevance in both natural and agricultural systems. Integrating comammox-related processes into soil and nutrient management strategies may therefore support more sustainable agricultural production while mitigating environmental impacts associated with nitrogen losses

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CHAPTER 12

ABIOTIC STRESS STUDIES CONDUCTED UNDER *IN VITRO* CONDITIONS IN VITICULTURE

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GÜLTEKİN ÖZDEMİR²

Introduction

The grapevine (*Vitis* spp.), whose evolutionary history dates back millions of years, is regarded as one of the earliest fruit species domesticated and cultivated by humans. Archaeological evidence indicates that the initial domestication of grapevine began approximately 6000–8000 years ago from wild populations of *Vitis vinifera* subsp. *sylvestris* in the Transcaucasian region situated between the Black Sea and Iran (Myles et al., 2011; Grassi and Arroyo-García, 2020). The most economically and agriculturally important grape species in the world is *Vitis vinifera* L. This species has a great diversity in terms of grape varieties and is valued as table, dried and wine grapes (This et al., 2006).

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Considered one of the foremost environmental threats of the 21st century, global climate change has led to numerous consequences, most prominently the sustained increase in average global temperatures. Furthermore, it is predicted that this change will cause major alterations in hydrological processes and energy cycles on a global scale (Droulia and Charalampopoulos, 2021). Climate change can have significant impacts on many areas and agricultural production worldwide. Viticulture is among the agricultural production activities significantly impacted by global climate change. Among the primary impacts of global climate change on grapevine are changes in the plant's phenological development stages. Because grapevine susceptibility to elevated temperatures is strongly dependent on its phenological stage, increases in temperature have the potential to markedly shift the timing of annual phenological events (Venios et al., 2020).

The adverse effects of global climate change on plant production systems have made it increasingly important to investigate abiotic stress factors, particularly drought and high temperatures, in greater detail and to develop plant materials with enhanced resistance to these conditions (Terán et al., 2024). A significant portion of research on abiotic stresses is conducted *in vivo*. However, such studies are costly and involve various limitations in terms of labor and time. Furthermore, the inability to control environmental factors can lead to risks that may affect the reliability of research results. Therefore, the use of *in vitro* techniques in research aimed at evaluating stress conditions is becoming increasingly common. *In vitro* studies conducted in controlled laboratory environments allow for more accurate and reproducible results by minimizing the impact of environmental variables. The use of tissue culture methods provides substantial advantages in the rapid and effective evaluation of stress tolerance, as well as in the characterization of physiological and biochemical

responses occurring under stress conditions. Furthermore, this method is considered a more cost-effective and efficient approach compared to studies conducted under external environmental conditions. Conducting abiotic stress research under *in vitro* conditions contributes to more effective use of the vegetation period, facilitates physiological examinations under controlled conditions, and accelerates the development process by enabling early selection applications in breeding programs (Bilir Ekbiç et al., 2022; Wijerathna-Yapa and Hiti-Bandaralage, 2023; Şaşkın et al., 2026). In recent years, tissue culture-based *in vitro* approaches have been widely adopted for the selection and development of stress-tolerant genotypes owing to their effectiveness and cost-effectiveness (Rai et al., 2011).

The aim of this section is to compile and evaluate research conducted under *in vitro* conditions on abiotic stress factors that cause significant yield and quality losses in viticulture. Within this scope, studies conducted on grapevines and grapevine rootstocks under drought, salinity, and high-temperature stress conditions were reviewed. The effects of these stress factors on plants, as well as the *in vitro* approaches employed to assess stress tolerance, were also discussed. Furthermore, based on the findings obtained, an attempt was made to highlight the importance of *in vitro* techniques in investigating stress tolerance and identifying resistant genotypes in viticulture.

Drought Stress

Drought represents a significant abiotic stress factor that restricts plant growth and development and consequently results in yield reduction. In response to drought stress, plants activate various morphological, physiological, and biochemical mechanisms to mitigate its detrimental effects. Drought stress causes wilting and yellowing of leaves in grapevines. Furthermore, as a defense mechanism against drought stress, plants close their stomata and

accumulate adaptive soluble compounds to maintain low water potential. Drought stress can result in elevated levels of reactive oxygen species in cells, leading to membrane lipid peroxidation, chlorophyll degradation, disruption of enzyme activities, and protein oxidation and aggregation (Siddique et al., 2016; Jiao et al., 2023). *In vitro* studies conducted under controlled laboratory conditions are widely employed to gain a deeper understanding of the adverse effects of drought on plants and to identify drought-tolerant genotypes (Shirazi et al., 2020; Mohsen et al., 2020). In *in vitro* culture systems, drought stress is generally artificially induced by reducing the water potential of the nutrient medium. To impose osmotic stress under experimental conditions, compounds such as polyethylene glycol (PEG), mannitol, and sorbitol are commonly utilized. These compounds artificially induce water-deficit conditions in tissue culture media, thereby enabling the investigation of the morphological, physiological, and biochemical responses developed by plants in response to drought stress (Ekinici et al., 2024). These studies, conducted under controlled conditions, offer significant advantages in revealing stress tolerance mechanisms and identifying drought-resistant genotypes early (Bilir Ekbic et al., 2022).

Studies on drought stress have been conducted under *in vitro* conditions. In this *in vitro* study, the responses of 'White Seedless' and 'Flame Seedless' grape varieties to drought stress induced by 0%, 0.5%, 1%, and 2% PEG 6000 concentrations were evaluated. Evaluation of the morphological, physiological, and biochemical traits indicated that the 'Flame Seedless' cultivar exhibited superior tolerance to drought stress relative to the 'White Seedless' cultivar. Furthermore, it has been reported that increasing PEG concentration significantly promotes proline accumulation in both varieties (Shirazi et al., 2020). To investigate the responses of different grapevine rootstocks to drought stress, Ramsey, 1103P, 140 Ru, and

Dogridge rootstocks were subjected to PEG 6000-induced drought conditions (0%, 1.5%, 3%, 4.5%, and 6%). As PEG concentration increased, morphological and physiological traits were adversely affected, whereas total proline accumulation, leaf abscission percentage, and drought severity index exhibited significant increases. The results revealed that the Ramsey rootstock exhibited the highest level of tolerance to drought stress, whereas Dogridge was identified as the most sensitive rootstock. The 1103P and 140 Ru rootstocks demonstrated intermediate tolerance to drought stress (Mohsen et al., 2020). Ghorbani et al. (2020) investigated the effects of manganese sulfate application on morpho-physiological characteristics and SOD activity in 'Thompson Seedless' and 'Rotabi' grape varieties under *in vitro* drought stress induced by PEG 6000. Drought stress led to a decline in growth parameters, including plant height, stem dry weight, leaf area, number of leaves and RWC; while manganese sulfate applications significantly reduced these negative effects. Furthermore, it was observed that the highest superoxide dismutase activity was recorded in the 'Rotabi' variety under severe drought conditions with increasing manganese levels. Bilir Ekbiç et al. (2022a) induced artificial drought stress with sorbitol in single-node *in vitro* conditions in the Balıkçı Siyahı (*Vitis labrusca* L.) grape variety. This study investigated the effects of various sorbitol doses on plant growth, and it was determined that increasing sorbitol concentrations caused leaf damage and wilting, as well as necrosis in shoots and stems. Increasing sorbitol dose resulted in a decrease in plant vitality, leaf number, plant height, node number and fresh weight and dry weight values. Furthermore, it was reported that 0.3 and 0.4 M sorbitol applications were more efficient in creating drought stress. Faizy et al. (2024) evaluated the responses of 'Superior' and 'Red Globe' grape varieties to drought stress *in vitro* in terms of morphological and biochemical parameters. It was reported that PEG-induced drought stress led to a significant increase in antioxidants (anthocyanin, proline, total sugars, and

catalase enzyme) produced in the explant; conversely, it resulted in a significant decline in malondialdehyde and chlorophyll content of the explant. The results showed that the 'Red Globe' variety is more resistant to drought stress than the 'Superior' variety and may be a more suitable variety for cultivation in water-scarce regions. In a study by Carvalho et al. (2024), the morphological and cytogenetic responses of 'Touriga Franca', 'Touriga Nacional', and 'Viosinho' grape varieties to *in vitro* water stress induced by PEG were investigated. In the study, moderate and severe water stress was applied, and it was determined that the stress negatively affected plant growth. However, differential tolerance levels were observed among the cultivars, with 'Viosinho' exhibiting reduced sensitivity to induced moderate and severe water stress, as reflected by its comparatively lower growth inhibition. It was reported that 'Touriga Franca' and 'Touriga Nacional' varieties adapted better to drought conditions in terms of leaf cell division and growth characteristics compared to the 'Viosinho' variety. In a study evaluating the effects of different salicylic acid concentrations under *in vitro* drought stress conditions induced by sorbitol in the Kober 5 BB grapevine rootstock, the addition of 2 mM SA to the culture medium was reported to have a positive effect in alleviating the adverse impacts of drought stress. Under artificial drought conditions induced by the highest sorbitol concentration, the application of 1 and 2 mM salicylic acid was found to alleviate the detrimental effects of drought stress, as indicated by improvements in damage severity, tolerance rate, ion leakage, cell membrane damage, and relative water content (Bilir Ekbiç and İlhan, 2024). Praveena et al. (2025) evaluated the responses of the grapevine cultivars 'Pusa Navrang', 'Pusa Swarnika', 'Perlette', and 'Male Hybrid', as well as the rootstocks 110R, St. George, Dogridge, and *Vitis parviflora*, under *in vitro* drought stress conditions induced by PEG-6000 supplementation of the culture medium. The findings revealed that PEG-induced drought stress markedly influenced the growth

performance and biochemical attributes of the eight *Vitis* genotypes. Explants cultured on PEG-supplemented media exhibited reduced LRWC and enhanced osmotic stress, leading to increased accumulation of compatible osmolytes, including proline and total soluble sugars.

High Temperature Stress

Temperature represents one of the key environmental factors that directly influence the physiological processes, growth, and developmental performance of grapevine. For viticulture to be economically viable in a region, the average annual temperature must be above 9 °C, the average temperature of the warmest month must be above 18 °C, the average temperature of the coldest month must be above 0 °C, the average temperature of the summer months must be above 20 °C, and the average temperature during the growing season must be above 13 °C. Regions with an average annual temperature between 11-16 °C are considered the most favorable areas for viticulture (Çelik et al., 1998). A threshold temperature of approximately 10 °C is needed for the vegetation period to begin in the grapevine. However, temperatures exceeding optimum levels during the sensitive stages of development; Photosynthesis, grape size, sugar accumulation, and the ripening process can be negatively affected by high temperatures (Soltekin et al., 2021; Santos and Figueiredo, 2023; Carreiras et al., 2023). Extreme temperatures are among the major abiotic stress factors that adversely affect the growth, development, and productivity of grapevine (Venios et al., 2020). Studies aimed at determining the responses of grapevines to high temperatures reveal the effects of different temperature levels and exposure times on plant development. In this context, ‘Narince’, ‘Çalkarası’, and ‘Öküzgözü’ grapevine cultivars were used as plant materials in an *in vitro* study aimed at determining appropriate temperature regimes and stress exposure durations for evaluating heat stress responses. The plantlets

grown under *in vitro* conditions were subjected to temperatures of 35°C and 40°C. For stress application at 35°C, the ambient temperature was gradually increased from 25°C to 35°C over 5 hours. The time when the temperature reached 35°C was considered the starting time, and the explants placed in the plant growing chamber were monitored every 6 hours. For stress application at 40°C, the ambient temperature was gradually increased from 25°C to 40°C over 7 hours. The study concluded that the optimal stress application time at 35°C was 36 hours, and at 40°C, 12 hours of stress application was appropriate (Altıncı et al., 2018).

Salinity Stress

Salinity stress is a major environmental problem that arises from the accumulation of high concentrations of soluble salts in the surface or deeper layers of the soil profile. Based on their salinity levels and pH values, soils are commonly classified into three distinct groups. Accordingly, soils with a salt content below 3‰ and a pH value between 7.1 and 8.5 are defined as mildly saline; those with a salt content between 3 and 6‰ and a pH value between 8.5 and 9.5 are moderately saline; and those with a salt content above 6‰ and a pH value exceeding 9.5 are defined as severely saline soils. Salinity stress results predominantly from the accumulation of neutral salts, particularly NaCl and Na₂SO₄, in the soil environment. Under these stress conditions, sodium ions are transported into plant cells via various channels and carrier proteins located in the cell membrane. Excessive sodium accumulating inside the cell disrupts the ion balance, leading to toxic effects. In addition, high extracellular salt concentrations reduce the osmotic potential of the environment, causing water to move out of the cells. As a result, water uptake by plant roots becomes difficult, leading to a stress condition called physiological drought (Oster et al., 1999; Kaiwen et al., 2020; Fang et al., 2021; Sharma et al., 2024; Şaşkın et al., 2026). The tolerance level of grapevine genotypes to salinity stress varies

depending on the species and variety. However, grapevine is generally regarded as a crop species with moderate sensitivity to elevated soil salinity levels. Furthermore, it is reported that *Vitis vinifera* L. species shows better tolerance to sodium chloride (NaCl)-induced salinity conditions compared to American grapevine species (Çelik et al., 1998). In studies aimed at determining plant tolerance to salinity stress, *in vitro* tissue culture methods offer significant advantages. These methods allow for precise control of environmental and experimental conditions, while also enabling the simultaneous evaluation of numerous genotypes in a limited area. Therefore, tissue culture techniques are widely preferred in research on salinity stress (Mozafari et al., 2018).

In an *in vitro* study evaluating the responses of the grapevine cultivars 'Çavuş', 'Müşküle', and 'Sultani Çekirdeksiz' to salinity stress, it was determined that increasing NaCl concentration and exposure duration reduced proliferation, growth, total chlorophyll content, and explant viability, while also inducing necrosis in the explants. Furthermore, 'Çavuş' was reported to be the most salt-tolerant cultivar, followed by 'Sultani Çekirdeksiz' and 'Müşküle' (Sivritepe and Eriş, 1999). In another study, 'Cardinal', 'Delight', 'Beauty Seedless', 'Pusa Seedless', 'Perlette', and 'Pearly of Csaba' grape varieties were exposed to NaCl (0, 25, 50, 75, 100, 125, 150, 175, and 200 mM)-induced salinity stress *in vitro*. The study reported that the salinity tolerance of the grape varieties was ranked as follows: Perlette>Pusa Seedless>Beauty Seedless >Delight > Pearly of Csaba > Cardinal (Singh et al., 2000). In a study determining the tolerance of Syrian native grape varieties (Ashlamesh, Helwani, Kassofee, and Khoudeiry) to salt (0, 10, 20, 30, 40, 80, 120, and 150 mM NaCl), it was reported that all vine varieties showed tolerance to NaCl up to 40 mM, while the application of 80 mM NaCl reduced chlorophyll content in most varieties and root number in all varieties. Furthermore, it was determined that 'Kassofee' and 'Khoudeiry'

showed lower sensitivity to NaCl among the four varieties (Charbaji and Ayyoubi, 2004). Mozafari et al. (2018), the 'Khoshnaw' grape variety was used as plant material. Plantlets were cultured on media supplemented with three salinity levels (0, 50, and 100 mM NaCl), three concentrations of iron nanoparticles (0, 0.08, and 0.8 ppm), and three concentrations of potassium silicate (0, 1, and 2 mM). The study reported that salinity stress increased ROS production, similar to hydrogen peroxide, and increased proline and total protein content in parallel with an increase in enzymatic antioxidant activity. Furthermore, it was determined that iron nanoparticle and potassium silicate applications significantly increased free proline and total protein content and enzymatic antioxidant activity, while decreasing hydrogen peroxide content. In a study conducted by Kassa and Feyissa (2020), the grapevine cultivars 'Chenin Blanc' and 'Canonannon' were transferred to culture media containing different concentrations of NaCl. The study results showed that as the NaCl concentration increased, the shoot and root number, shoot and root length, leaf and node number, node length, and fresh and dry weights of roots and shoots reduced significantly. Furthermore, it was reported that the 'Canonannon' variety was more tolerant to NaCl than the 'Chenine Blanc' grape variety. In another study conducted under *in vitro* conditions, plantlets of the Balıkcı Siyahı (*Vitis labrusca* L.) grape variety were transferred to nutrient media containing four different concentrations (0, 0.5, 1, 2 mM) of H_3BO_3 and three different concentrations (0, 100, 200 mM) of NaCl. The study found that the application of 200 mM salt negatively affected growth and development parameters. Furthermore, it was reported that 0.5 mM and 1 mM H_3BO_3 concentrations were more effective in alleviating salt stress, whereas the application of 2 mM H_3BO_3 caused toxic effects (Akden et al., 2024).

Conclusion

Sustainable viticulture is increasingly threatened by the growing incidence of environmental stresses, including water limitation, thermal stress, and salinity, driven by ongoing climatic changes. Identifying varieties and rootstocks resistant to these stress factors, which directly affect the yield and quality characteristics of the grapevine, is among the goals of current viticulture research. *In vitro* culture techniques, developed for this purpose, offer significant advantages in comparing genotypes and determining tolerance levels by allowing the creation of stress conditions in a controlled environment. The research discussed in this section highlights the effectiveness of *in vitro* methodologies in assessing the impacts of environmental stress factors, including drought, salinity, and high temperature, on grapevine. Studies employing stress-inducing agents such as PEG, sorbitol, and NaCl have facilitated the evaluation of diverse grapevine cultivars and rootstocks under standardized conditions, thereby enabling the reliable identification of genotypic differences in stress tolerance. One of the principal advantages of *in vitro* techniques is their capacity to allow the rapid screening of a large number of genotypes under strictly controlled environmental conditions. Furthermore, the use of these techniques allows for year-round production regardless of the vegetation period. In conclusion, *in vitro* stress research can be considered an important preliminary assessment tool in studies aimed at developing resistant varieties and rootstocks in viticulture. In the future, the widespread use of integrated research combining physiological, biochemical, and molecular approaches will facilitate a more detailed understanding of stress tolerance mechanisms. Therefore, the incorporation of *in vitro* culture techniques into modern breeding programs may provide valuable opportunities for alleviating the detrimental effects of climate change on viticulture and enhancing the sustainability of grape production systems.

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CHAPTER 13

DIGITALIZATION IN TEXTILES AND SUSTAINABLE FIBER INTEGRATION

SUAT CUN¹

Introduction

The textile and apparel sector, one of the most dynamic global drivers of the twenty-first century, generates substantial economic value along the supply chain from raw material procurement to final products, while simultaneously standing at the threshold of a fundamental paradigm shift in terms of raw material quality standardization and traceability (Niinimäki et al., 2020). The competitive dynamics of the global market, international regulatory frameworks, and industrial quality standards necessitate the digitalization of all processes ranging from the production of natural fibers—the first link in the textile value chain—to yarn testing laboratories (Elijah et al., 2023). The primary objective of this technological transformation is to optimize the fiber quality of strategic industrial crops such as cotton (*Gossypium hirsutum* L.) and

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flax (*Linum usitatissimum* L.), which constitute the main input of the textile industry, through artificial intelligence, automation, and data analytics systems (Gries et al., 2015).

In the natural fiber industry, achieving the high raw material standards required by the textile sector (fiber length, strength, uniformity, and micronaire) is directly related to the precision of both pre-harvest agronomic practices and post-harvest operations (Constable and Bange, 2015). Traditional raw material procurement, classification, and logistics methods are insufficient to provide the real-time data flow and quality optimization required by modern industrial systems (Raza et al., 2019). At this point, the integration of Precision Agriculture and Textile 4.0 enables the digital monitoring of crop development in the field using unmanned aerial vehicles (UAVs/drones) and multispectral satellite imaging technologies (Mulla, 2013), thereby making it possible to remotely estimate the technological properties of unharvested fibers through remote sensing indices (Tsouros et al., 2019).

The industrial counterpart of this system integration is reflected in automation solutions in ginning facilities and big data analytics in yarn testing laboratories (Kagermann et al., 2013). Large multivariate datasets generated from advanced fiber testing instruments such as HVI (High Volume Instrument) and AFIS (Advanced Fiber Information System) have evolved beyond simple statistical reports and have become the core input for advanced digital decision-support systems (Kumar, 2018). Processing these laboratory outputs using mathematical and chemometric modeling approaches such as Analysis of Variance (ANOVA) and Principal Component Analysis (PCA biplots) provides textile engineers with the capability to develop optimal blending recipes, predict spinning performance using artificial neural networks, and minimize downtime in production lines (Qin et al., 2016). Furthermore, the entire lifecycle of raw materials, digitally identified via RFID and

barcode technologies from field to end consumer, is recorded through blockchain architecture, thereby establishing a transparent and reliable traceability matrix in the global market (Agrawal et al., 2018; Choi, 2019).

Textile 4.0: Core Components of Digital Transformation in the Textile Industry

Textile 4.0, defined as the adaptation of the Industry 4.0 paradigm to the textile and apparel manufacturing sector, transforms traditional and labor-intensive production lines into dynamic, flexible, and self-optimizing smart factories through cyber-physical systems (CPS) (Gries et al., 2015). This digital transformation is based on a set of integrated vertical and horizontal technological components aimed at minimizing inefficiencies across all stages, from heterogeneous agricultural raw material inputs (such as cotton, flax, etc.) to final garment products (Alcácer and Cruz-Machado, 2019). The core components forming the architecture of Textile 4.0 are classified as the Internet of Things (IoT), cyber-physical systems, cloud computing, artificial intelligence (AI), and big data analytics (Kumar, 2018).

The first fundamental component of Textile 4.0, which connects the physical and digital worlds, is the Internet of Things (IoT) and advanced sensor networks. Smart sensors integrated into fiber preparation, ginning, carding, spinning, and weaving machines continuously monitor process parameters such as temperature, humidity, tension, speed, and raw material flow rate in real time (Elijah et al., 2018; Shishoo, 2012). The real-time data obtained from these sensors are transferred to the digital environment via cyber-physical systems (CPS), enabling production machines to communicate with each other as well as with central control units (Monostori et al., 2016). In this way, an instantaneous change in fiber strength or a deviation in yarn tension is detected within milliseconds by machine automation, and operational parameters are

autonomously adjusted without the need for human intervention (Ahuett-Garza and Kurfess, 2018).

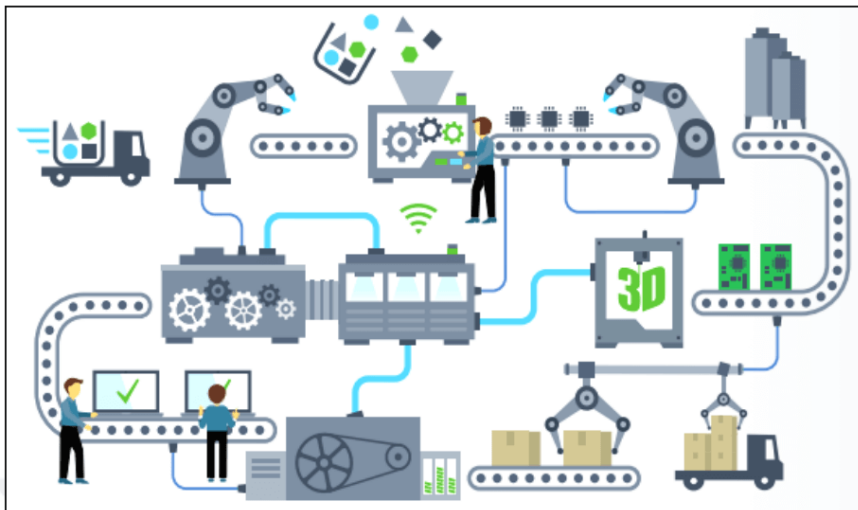


Figure 1. Industry 4.0: digital and interconnected manufacturing

The consolidation of quality parameters from raw material laboratories in textile enterprises (HVI and AFIS datasets) with production line performance data in the cloud provides companies with a macro-level operational visibility (Zheng et al., 2018).

The most critical complementary components of the Textile 4.0 paradigm at the supply chain level are *Digital Twin* technology and advanced integration software systems (Verdouw et al., 2021). Creating a digital replica of a physical textile factory or raw material flow chain enables the early identification of potential bottlenecks through simulation-based analyses (Tao et al., 2018).

The Strategic Importance of Sustainable Natural Fibers

Although fossil fuel-based synthetic fibers (such as polyester, nylon, and acrylic) have long dominated the global textile market due to their low production costs and high manufacturing volumes, this dominance has generated a significant ecological crisis

(Niinimäki et al., 2020). The high carbon intensity of synthetic fiber production and the microplastic pollution released during their washing processes cause irreversible damage to both marine and terrestrial ecosystems (Henry et al., 2019; Boucher and Friot, 2017). In addition to these environmental impacts, global regulatory frameworks such as the European Green Deal and circular economy action plans are compelling the textile industry to fundamentally transform its raw material strategies (Niinimäki et al., 2020). In this context, sustainable natural fibers such as cotton (*Gossypium hirsutum* L.) and flax (*Linum usitatissimum* L.), with their biodegradable nature, renewable resource base, and low carbon footprint potential, have become strategic components of the textile value chain (Muthu, 2020).

Cotton, the flagship crop of the natural fiber industry, plays a vital role in global agricultural trade and employment (Meyer and Dew, 2021). However, the extensive use of pesticides and chemical inputs in conventional cotton cultivation has raised serious concerns regarding its environmental sustainability. In contrast, cotton fibers produced under sustainable standards not only help preserve soil ecological health but also enable the maintenance of key technological fiber properties—such as fiber length and strength—even under the conditions imposed by climate change (Lu et al., 2015).

Another strategic natural raw material in the textile value chain is flax fiber (*Linum usitatissimum* L.). From an agronomic perspective, flax is a climate-smart industrial crop with a significantly lower input requirement compared to cotton—particularly in terms of water consumption and crop protection chemicals—and it exhibits a high level of adaptability even in marginal growing areas (Stavropoulos et al., 2023). The high tensile strength, moisture absorption capacity, and natural antimicrobial properties of flax fibers elevate them from being used solely in

apparel applications to a premium and strategic raw material for technical textiles and green composite manufacturing (Pickering et al., 2016).

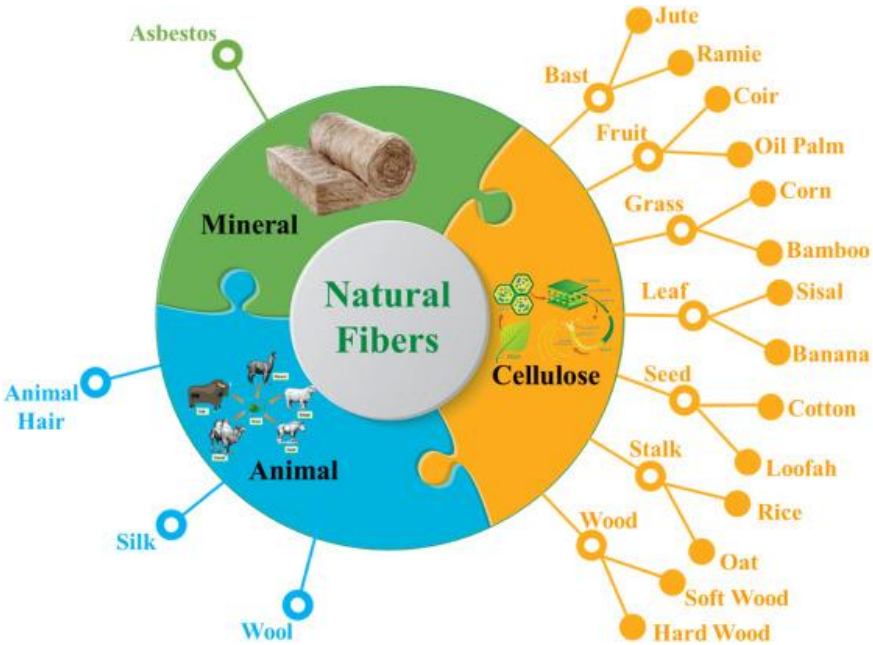


Figure 2. Classification of fibers according to their origins (Elfaleh et al., 2023)

This indicates that the strategic importance of sustainable natural fibers does not derive exclusively from their biodegradability; rather, it is also rooted in their ability to provide a high-quality, traceable, and eco-ethical compliant industrial raw material base for the increasingly digitalized textile industry (Textile 4.0) (Choi, 2019).

Automation in Ginning and Pre-Processing Technologies

The first and most critical industrial stage in which harvested seed cotton or fibrous industrial crops are processed before being supplied to textile mills as raw material is ginning and pre-processing operations (USDA, 2022). Although ginning is fundamentally the process of separating fiber from the seed

(cottonseed), it represents a critical threshold in terms of modern textile raw material standards, where fiber quality (length, strength, short fiber content) can either be preserved or damaged due to aggressive mechanical processing (Hake et al., 1996). In traditional ginning facilities, when the heterogeneous nature of raw material inputs (e.g., moisture content, foreign matter levels) is processed under fixed machine settings, significant mechanical fiber damage, breakage, and neps (fiber neps) may occur (USDA, 2022). To mitigate these agronomic and industrial variations, smart gin automation systems dynamically optimize the process through cyber-physical systems and sensor networks (Qin et al., 2016).

At the core of modern automated ginning systems are real-time moisture and color sensors installed along the raw material flow line, as well as microwave and infrared spectroscopy-based measurement devices (Zumba et al., 2017). These smart sensors, which measure the instantaneous moisture content and foreign matter (trash) intensity of seed cotton upon arrival at the facility, transmit data to cyber-physical control units (Pelletier and Byler, 2020). If the incoming raw material contains high moisture levels, the system autonomously optimizes the temperature of drying towers; if moisture is within the optimal range, drying units are deactivated to prevent excessive heating of the fiber and thus avoid strength loss (Baker, 2012).

Another technological dimension of automation in the ginning process is the digitalization of post-harvest logistics and raw material traceability (Wang et al., 2024). After the ginning process is completed, the fibers, which are compressed into bales using hydraulic presses, are transferred to automated weighing and packaging lines. At this stage, Radio Frequency Identification (RFID) tags and smart barcode systems integrated into each cotton or flax bale assign a permanent “digital identity” to the raw material (Agrawal et al., 2018).

This digital identity is linked with bale weight, ginning outturn, processing facility parameters, and laboratory quality classifications (HVI data) and subsequently recorded in cloud-based databases. Automation solutions in ginning and pre-processing technologies not only transform heterogeneous agricultural inputs into a homogeneous and standardized industrial raw material but also establish the foundational infrastructure for the digital traceability systems required by textile enterprises (Gries et al., 2015; Choi, 2019).



Figure 3. A view of a cotton processing facility

Smart Ginning Systems and Yield Automation

The most fundamental indicator of economic sustainability and operational performance in the ginning industry is ginning outturn. Ginning outturn refers to the proportional ratio of lint cotton obtained from seed cotton entering the gin, and it is directly associated with producer income and the raw material cost of spinning mills (ICAC, 2021; El-Feky, 2010). In traditional saw-gin or roller-gin systems, yield is largely managed based on fixed mechanical settings and operator experience (USDA, 2022; Hake et

al., 1996). However, variations in raw material characteristics (such as varietal properties, 100-seed weight, and seed structure) processed under constant mechanical pressure may lead either to fiber breakage and yield losses or to seed coat fragments contaminating the fiber as a result of seed damage (Armijo et al., 2009).

In modern smart ginning systems, yield automation is based on real-time monitoring of the mathematical relationship between the feed rate of the gin stand and the motor torque of the saw or roller shaft (Gillum, 1983). Smart load cells and current sensors integrated into the gin body measure the resistance and mechanical load encountered during fiber–seed separation in real time (Sui and Byler, 2012; NCGA, 2021). Advanced proportional–integral–derivative (PID) controllers and fuzzy logic algorithms process these data to optimize the speed of the feed rollers within milliseconds (Kumar, 2018).

Role of Sensor Technologies in Preventing Fiber Damage During the Ginning Process

The ginning process is, by its nature, an aggressive operation in which fibers are exposed to high-speed mechanical forces, representing one of the most sensitive stages in the textile value chain in terms of preserving fiber quality (Hardin et al., 2018). In saw-gin or roller-gin systems, uncontrolled mechanical stress applied during the separation of fiber from seed and subsequent removal of foreign matter leads to fiber breakage, an increase in short fiber index (SFI), and neps formation, one of the most critical defects in the spinning industry (Whitelock et al., 2019). Such fiber damage directly reduces spinning performance in yarn manufacturing and negatively affects the strength and dyeability of the final textile product (Kelly et al., 2015). The prevention of these microscale mechanical damages critically depends on next-generation smart sensor technologies and cyber-physical feedback control loops (Hardin et al., 2018; Whitelock et al., 2019).

The most important sensor architecture for minimizing fiber damage in the ginning line is real-time near-infrared (NIR) and microwave-based moisture sensing systems (Byler, 2006). Smart moisture sensors continuously measure the moisture content of fibers along the processing line and dynamically control drying and conditioning units, ensuring that fibers remain within an optimal moisture range (approximately 6.5%–7.5%) where they exhibit maximum elasticity against mechanical stress. This sensor-driven moisture regulation creates a protective buffer that enables mechanical lint cleaners to operate without causing fiber damage (Hardin et al., 2018).

The prevention of fiber damage during the mechanical cleaning stage is further enhanced through computer vision and laser-based scanning technologies. High-speed industrial cameras and optical sensors installed along the fiber flow line enable real-time analysis of trash content, seed coat fragments, and neps density in processed fibers (Zhang and Li, 2014). Advanced artificial intelligence algorithms and multivariate statistical models process these optical data to continuously assess fiber damage risk (Hardin et al., 2018). If optical sensors detect that short fiber ratio or neps count exceeds predefined thresholds, the cyber-physical system automatically activates bypass valves to divert fibers away from aggressive saw-type cleaners or autonomously reduces the rotational speed of cleaning cylinders (van der Sluijs, 2015; Hardin et al., 2018). Thus, multi-sensor networks integrated into the ginning process not only clean the raw material but also maintain mechanical forces within the physical tolerance limits of plant fiber anatomy, ensuring comprehensive protection of fiber quality (Whitelock et al., 2019).

Conclusion

Although the current state of Textile 4.0 components and the integration of sustainable natural fibers has already opened the door

to a data-driven era in the industry, the future perspective of these technologies points toward a far more radical industrial transformation. The textile value chain of the future will encompass not only production in the field or automation in factories, but a fully circular (closed-loop) eco-digital system in which raw materials move from soil to runway and then back to the field. At the core of this future lies the synergy between artificial intelligence (AI) and circular economy models. AI-powered generative models and machine learning algorithms will not only predict fiber quality, but will also simultaneously analyze global climate simulations, textile waste recycling data, and consumer trends, thereby autonomously determining which crop varieties with specific agronomic traits (e.g., fiber length, strength, etc.) should be cultivated in the field.

However, despite this progress, several sectoral challenges and limitations still hinder the large-scale global adoption of a fully integrated and sustainable raw material supply chain within the Textile 4.0 framework.

Addressing these constraints and enabling the digitalization of sustainable fiber engineering will require the implementation of macro-level strategic policy frameworks. In conclusion, digitalization in textiles and the integration of sustainable fibers should not be interpreted merely as a mechanical modernization process or a green marketing strategy. Rather, this transformation is inevitable within the context of agricultural bioeconomy and industrial digitalization (Textile 4.0). This holistic approach—ranging from plant-based raw material engineering in the field, through ginning automation and multivariate laboratory data analytics (ANOVA and PCA biplots), to blockchain-based transparency—represents the only viable vision that will enable the textile industry to survive within ecological boundaries while simultaneously achieving a high-value, traceable, and globally competitive production standard.

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CHAPTER 14

OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE SYSTEMS IN PLANTS: STRESS ADAPTATION AND YIELD STABILITY IN INTEGRATED AGRICULTURAL SYSTEMS

ZEMZEM FIRAT¹

Introduction

Environmental systems encompass highly complex and dynamic habitats in which humans, animals, plants, and a wide range of macro- and microorganisms coexist and interact (Saxena, 2025). As a consequence of these interactions, plants are continuously exposed to numerous environmental challenges. Due to their sessile nature, they are particularly vulnerable to abiotic stress factors such as drought, salinity, extreme temperatures, and oxidative stress. These adverse conditions can severely impair plant survival, growth, development, and productivity (Zhang et al., 2022). Environmental stresses frequently disrupt cellular homeostasis and ionic balance, leading to substantial physiological and metabolic disturbances in plants (Rajput et al., 2021).

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In contrast, biotic stresses arise from living organisms, including pathogens, insects, and other pests that negatively affect crop performance and induce disease development (Gimenez et al., 2018). The occurrence of either biotic or abiotic stress at any stage of plant development can markedly influence plant growth and developmental processes (Javed et al., 2020). Such stress conditions often disturb cellular equilibrium and ion distribution, resulting in osmotic imbalance and enhanced generation of reactive oxygen species (ROS) (Herbette et al., 2011). Excessive ROS accumulation can cause severe damage to cellular organelles and impair their functions. Furthermore, elevated ROS levels promote membrane lipid peroxidation, disrupt cellular membranes, degrade biological macromolecules, and ultimately trigger cell death (Rajput et al., 2021).

Reactive oxygen species comprise a diverse group of oxygen-derived free radicals, reactive molecules, and ionic forms (Nafees et al., 2019). In plants, ROS exist in both ionic and molecular states. The ionic forms include hydroxyl radicals ($\bullet\text{OH}$) and superoxide anions ($\text{O}_2^{\bullet-}$), whereas the principal molecular forms are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$) (Mittler et al., 2004). These reactive compounds influence numerous physiological processes and may induce structural alterations as well as the degradation of essential cellular macromolecules under stress conditions (Huang et al., 2019).

Oxidative stress refers to a physiological state characterized by the excessive production of reactive species within living organisms (Sies and Jones, 2020). In plants, oxidative stress may result either directly from unfavorable environmental conditions or indirectly through the overproduction and accumulation of ROS. If these reactive compounds are not efficiently detoxified, they can progressively damage cellular structures and intensify stress-related injuries (Xie et al., 2019). Persistent ROS accumulation adversely

affects organelle integrity and cellular functionality while accelerating membrane peroxidation, macromolecular degradation, and programmed or uncontrolled cell death. To prevent excessive ROS-induced damage while preserving their crucial role as intracellular signaling molecules, plants have evolved sophisticated antioxidant defense systems that tightly regulate ROS homeostasis (Afzal et al., 2014).

Under optimal conditions, plant cells maintain a dynamic equilibrium between the production of reactive oxygen species (ROS) and antioxidant defense systems. Maintaining this balance is crucial for sustaining cellular redox homeostasis and ensuring the proper functioning of signaling pathways that regulate plant growth, development, and stress adaptation (Hasanuzzaman et al., 2020; Mittler et al., 2022).

This chapter examines plant responses to oxidative stress, focusing on its physiological and biochemical impacts, describing the antioxidant defense mechanisms involved, and evaluating the adaptive strategies that plants employ to minimize oxidative damage and maintain cellular functionality under adverse environmental conditions.

Basic Mechanisms of Oxidative Stress

Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) act as oxidative signaling molecules that are generated through both primary and secondary metabolic processes, conveying essential information about cellular metabolism and the energetic status of different intracellular compartments. Accordingly, ROS are considered key signaling components that contribute to cellular viability, adaptation, and survival under fluctuating environmental conditions (Ravi et al., 2023).

ROS are continuously produced in various cellular compartments, including mitochondria, chloroplasts, peroxisomes, and the plasma membrane, where they play important roles in a wide range of physiological processes and signaling pathways (Noctor et al., 2018).

Under unfavorable environmental conditions, however, ROS production may exceed the cellular detoxification capacity, leading to a phenomenon commonly referred to as an oxidative burst. Excessive accumulation of ROS can result in oxidative damage to proteins, lipids, nucleic acids, and other biomolecules, thereby disrupting cellular integrity and metabolic functions. Such damage negatively affects plant growth, development, productivity, and ultimately survival. Therefore, maintaining a tightly regulated balance between ROS generation and scavenging mechanisms is essential for preserving cellular homeostasis and ensuring the proper functioning of physiological processes in plants (Szechyńska-Hebda et al., 2022).

Superoxide Radical

The superoxide anion ($O_2^{\bullet-}$) is one of the primary reactive oxygen species generated through the one-electron reduction of molecular oxygen and commonly serves as a precursor for other ROS, especially hydrogen peroxide (H_2O_2). Under normal physiological conditions, intracellular levels of $O_2^{\bullet-}$ are considerably lower than those of H_2O_2 . Moreover, the generation of $O_2^{\bullet-}$ is frequently linked to increased accumulation of H_2O_2 , since two superoxide molecules can react with two protons (H^+) to form hydrogen peroxide (Wang et al., 2023).

Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) is a non-radical reactive oxygen species (ROS) whose intracellular levels are tightly regulated, generally maintained within the nanomolar to micromolar range

(10^{-5} – 10^{-9} M) (Szechyńska-Hebda et al., 2022). However, under specific environmental or physiological conditions, H_2O_2 levels in plants may rise to higher concentrations, reaching approximately 10^{-5} – 10^{-4} M (Asaeda et al., 2022).

Hydroxyl Radical ($\bullet\text{OH}$)

In general, ROS that possess radical structures are more reactive than non-radical ROS. Among them, the hydroxyl radical ($\bullet\text{OH}$), which contains a single unpaired electron, is considered the most reactive form of ROS. Due to its extremely high reactivity, it can induce structural modifications in proteins, trigger lipid peroxidation, and cause damage to cellular membranes (Wang et al., 2023).

Singlet Oxygen ($^1\text{O}_2$)

Singlet oxygen ($^1\text{O}_2$) corresponds to the first excited electronic state of molecular oxygen (Wang et al., 2023). Under neutral conditions, its concentration is estimated to be around $\sim 10^{-13}$ M. The threshold level required to initiate membrane damage is approximately $\sim 10^{-8}$ M, while higher local concentrations in the range of $\sim 10^{-5}$ – 10^{-4} M are generally associated with the induction of cell death (Szechyńska-Hebda et al., 2022).

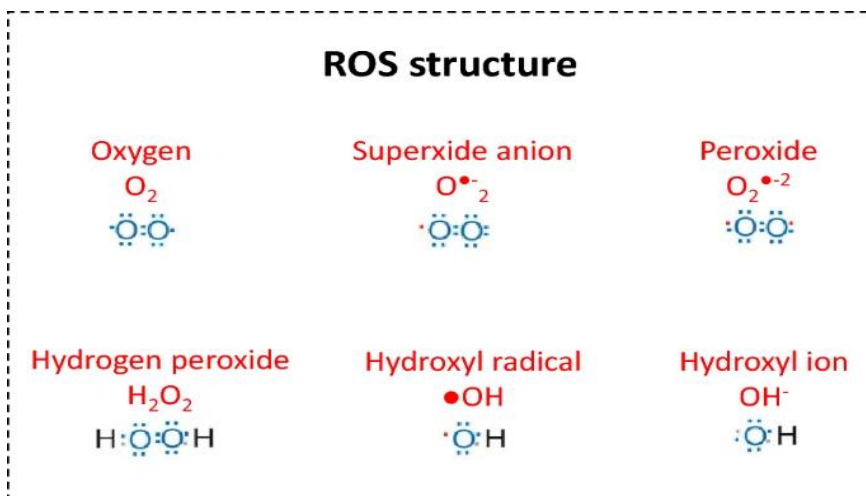


Figure 1. Structure of reactive oxygen species in plants

An uncontrolled increase in ROS levels leads to the progressive accumulation of oxidative damage in cellular components (Asada, 2006).

Effects of Oxidative Stress on Plant Metabolism

Exposure of plants to adverse environmental conditions can lead to excessive reduction of the electron transport chain, thereby promoting photooxidative damage and disrupting cellular redox balance (Gururani et al., 2015). Furthermore, environmental stresses negatively influence the ultrastructural organization of cellular organelles, alter the biosynthesis and accumulation of photosynthetic pigments, and affect the levels of essential metabolites and enzymatic components involved in plant metabolism. Collectively, these disturbances impair photosynthetic efficiency and restrict overall carbon assimilation, ultimately reducing plant growth and productivity (Ashraf and Harris, 2013).

In addition, certain abiotic stress factors, particularly elevated temperatures, can adversely affect the functionality of photosystem II (PSII), leading to a decline in photochemical

efficiency. High-temperature stress may also interfere with the regeneration of ribulose-1,5-bisphosphate (RuBP), thereby limiting the Calvin cycle and reducing photosynthetic performance (Fahad et al., 2017).

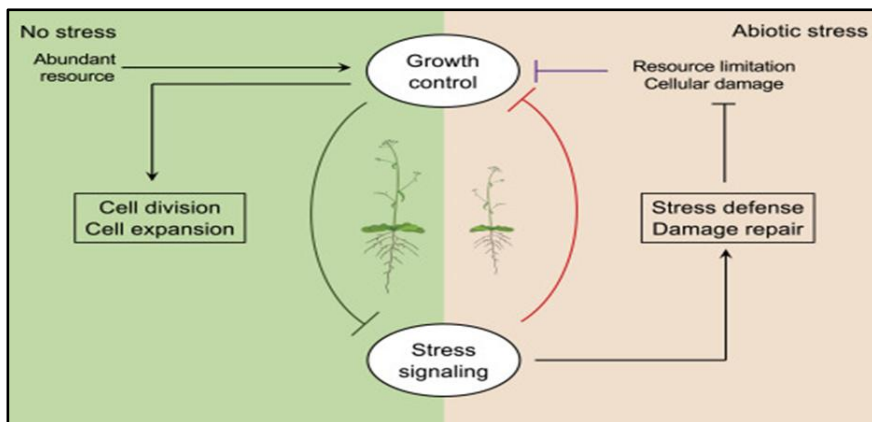


Figure 2. Relationship Between Stress Signaling and Plant Growth (Zhang et al., 2020)

Antioxidant Defense Mechanisms in Plants

By definition, antioxidants are molecules that can inhibit or delay reactions mediated by free radicals. These compounds play a vital role in protecting cells from damage by limiting oxidative processes. Notably, even at concentrations lower than those of potentially oxidizable substrates, antioxidants are able to effectively suppress or slow the progression of oxidation reactions (Dumont & Rivoal, 2019).

Plants have developed a broad range of defense mechanisms to cope with oxidative stress, including the synthesis and accumulation of antioxidant molecules and the activation of stress-responsive signaling pathways. These coordinated systems enable plants to maintain cellular redox homeostasis and reduce the

damaging effects of reactive oxygen species under adverse environmental conditions.

Enzymatic Antioxidants

Enzymatic defense systems against ROS comprise a variety of enzymes, including glutathione reductases, dehydroascorbate reductases, superoxide dismutases, monodehydroascorbate reductases, glutathione peroxidases, catalases, and ascorbate peroxidases (Mansur et al., 2022).

Superoxide Dismutases (SODs)

Superoxide dismutases (SODs; EC 1.15.1.1) are metalloenzymes present in virtually all aerobic organisms and constitute a primary line of defense against reactive oxygen species (ROS) at the cellular level. These enzymes catalyze the dismutation of the highly reactive superoxide anion ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), thereby contributing significantly to the reduction of oxidative stress and the maintenance of cellular redox equilibrium (Chung, 2017).

In plants, SOD isoenzymes are categorized based on the metal ion present at their catalytic centers. Accordingly, three main classes are recognized: copper/zinc superoxide dismutases (Cu/Zn-SOD), manganese superoxide dismutases (Mn-SOD), and iron superoxide dismutases (Fe-SOD) (Wang et al., 2016). These isoforms are selectively localized to different subcellular compartments, including chloroplasts, mitochondria, the cytosol, and peroxisomes, via specific N-terminal targeting signals. In these locations, they function cooperatively in ROS detoxification and collectively support the maintenance of intracellular oxidative balance (Bowler et al., 1992).

Catalase (CAT)

Catalase (CAT; EC 1.11.1.6) is an essential antioxidant enzyme found in all aerobic organisms, including plants, and is mainly localized in peroxisomes. It is a heme-containing tetrameric protein that plays a crucial role in the detoxification of hydrogen peroxide (H_2O_2), which is continuously produced during various metabolic activities. Considerable amounts of H_2O_2 are generated through processes such as fatty acid β -oxidation, mitochondrial electron transport, and photorespiration. By removing excess H_2O_2 , catalase prevents its accumulation to toxic levels and thereby protects cellular components from oxidative injury (Mhamdi et al., 2010).

Catalase catalyzes the conversion of hydrogen peroxide into water (H_2O) and molecular oxygen (O_2), thereby contributing to the maintenance of intracellular redox balance. Unlike many other peroxidase enzymes, this reaction does not require reducing power or external electron donors. This characteristic makes catalase a highly efficient and energetically favorable detoxification mechanism, particularly under conditions of elevated H_2O_2 production (Bowler et al., 1992). The enzyme is especially important in peroxisomes, where it facilitates the removal of H_2O_2 generated during photorespiratory metabolism.

Ascorbate Peroxidase (APX)

Ascorbate peroxidase (APX; EC 1.11.1.11) is an important antioxidant enzyme of the peroxidase family that uses ascorbate (AsA) as an electron donor to reduce hydrogen peroxide (H_2O_2) to water. It plays a key role in reducing the harmful effects of reactive oxygen species (ROS) and supporting the cellular defense system in plants (Maruta and Ishikawa, 2018).

APX is a core enzyme of the ascorbate–glutathione cycle, where it contributes to the efficient detoxification of H_2O_2 and helps

maintain intracellular redox balance. For this reason, it is regarded as one of the major enzymatic ROS-scavenging systems in higher plants. APX exists in several isoforms that are targeted to different subcellular compartments, such as chloroplasts, mitochondria, peroxisomes, and the cytosol (Ozyigit et al., 2016). This compartmentalization allows APX to effectively remove H_2O_2 produced in different parts of the cell.

Overall, APX is one of the key enzymatic defense components that enables plants to cope with ROS-induced oxidative stress, especially under unfavorable environmental conditions.

Dehydroascorbate Reductase (DHAR)

Dehydroascorbate reductase (DHAR; EC 1.8.5.1) is a key enzymatic antioxidant that plays an essential role in maintaining cellular redox homeostasis in plants. It contributes to the regeneration of the ascorbate pool in both the apoplastic and symplastic compartments, thereby helping to stabilize the intracellular redox environment (Khan & Khan, 2017).

DHAR catalyzes the conversion of dehydroascorbate (DHA) back into ascorbic acid (AsA) using reduced glutathione (GSH) as the electron donor. This reaction is a central step in the ascorbate–glutathione cycle, supporting the continuity of the antioxidant system and reducing oxidative stress caused by reactive oxygen species (ROS). Through this process, DHAR is crucial for maintaining redox balance and improving plant tolerance to environmental stress.

In addition, monodehydroascorbate reductase (MDHAR), predominantly located in chloroplasts, provides an alternative pathway for ascorbate regeneration by reducing monodehydroascorbate back to AsA (Shin et al., 2014). Together, DHAR and MDHAR operate in a coordinated manner to sustain the

cellular ascorbate pool and regulate redox homeostasis under both normal and stress conditions.

Glutathione Reductase (GR)

Glutathione reductase (GR; EC 1.6.4.2) is a flavoprotein oxidoreductase that is widely present across all kingdoms of life. It has a central function in sustaining the ascorbate–glutathione cycle, which is essential for maintaining cellular redox homeostasis. GR catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to its reduced form (GSH), thereby supporting the continuous functioning of the antioxidant defense system (Hasanuzzaman et al., 2017).

In plant cells, chloroplasts are the main site of GR activity, although the enzyme is also found in mitochondria and the cytosol, where it helps maintain redox balance within specific cellular compartments. As a low-molecular-weight flavoprotein reductase, GR uses NADPH as an electron donor to regenerate GSH, which is critical for sustaining antioxidant capacity under both normal and stress conditions.

Reduced glutathione (GSH) is a key component of the ascorbate–glutathione cycle, participating in the regeneration of ascorbic acid from dehydroascorbate (DHA) and monodehydroascorbate (MDHA). In this process, GSH is converted into GSSG, which is then recycled back to its reduced form by GR, ensuring the continuity of the antioxidant cycle (Foyer and Noctor, 2011). Overall, this system represents a fundamental defense mechanism for detoxifying reactive oxygen species (ROS) and maintaining cellular redox equilibrium.

Non-Enzymatic Antioxidants

Non-enzymatic antioxidants, including ascorbate, glutathione, carotenoids, tocopherols, and a wide range of phenolic

compounds, represent key components of the plant antioxidant defense system and play an important role in detoxifying reactive oxygen species (ROS) under environmental stress conditions. By doing so, they help reduce oxidative damage and protect cellular structures (Hasanuzzaman et al., 2020).

These metabolites function in a coordinated and synergistic manner within the antioxidant network. Major redox-active molecules include ascorbic acid (AsA), reduced glutathione (GSH), α -tocopherol, carotenoids, flavonoids, as well as plastoquinone and plastocyanin. In addition to their direct ability to scavenge ROS, these compounds are also crucial for maintaining intracellular redox balance and regulating signaling pathways involved in plant responses to oxidative stress (Hasanuzzaman et al., 2020).

Ascorbic Acid (Vitamin C)

Ascorbic acid, commonly known as vitamin C, is a well-characterized antioxidant that serves as a primary substrate in the detoxification of reactive oxygen species (ROS) (Akram et al., 2017). Its strong antioxidant potential is mainly related to its ability to donate electrons in both enzymatic and non-enzymatic redox reactions, as well as its capacity for regeneration, making it highly efficient in counteracting oxidative stress (Noctor and Foyer, 1998). In this way, ascorbate helps keep ROS levels within a non-toxic physiological range and protects cellular structures from oxidative damage (Shao et al., 2008).

In plant cells, ascorbic acid is predominantly synthesized through the Smirnoff–Wheeler pathway, which is closely associated with mitochondrial metabolism, while a smaller portion is produced via the D-galacturonic acid pathway (Ashraf et al., 2019). Owing to its distribution in both the cytosol and apoplast, ascorbic acid plays a vital role in protecting plant cells against ROS-induced injury and

in maintaining redox homeostasis under both normal and stress conditions.

Glutathione

Glutathione is a ubiquitous thiol-containing tripeptide (γ -glutamylcysteinylglycine) found in all aerobic organisms and is one of the most important low-molecular-weight antioxidants involved in cellular redox regulation. The enzyme glutathione reductase catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to its reduced form (GSH), thereby sustaining the cellular glutathione pool in its reduced state (Diaz-Vivancos et al., 2015).

Reduced glutathione has a protective function in maintaining the integrity of sulfhydryl (-SH) groups in enzymes and structural proteins against oxidative damage. It performs this role either through direct scavenging of reactive oxidants or via glutathione–disulfide exchange reactions that restore oxidized protein thiols, thereby preserving protein structure and function (Gill et al., 2013).

In addition to its antioxidant role, this non-enzymatic molecule is involved in the regulation of enzyme activity and participates in numerous cellular processes, including xenobiotic detoxification, cell division, differentiation, programmed cell death, and senescence. Glutathione also contributes to the synthesis of proteins, nucleotides, and phytochelatins, as well as metabolite conjugation and the regulation of stress-responsive gene expression (Zeng et al., 2017).

Carotenoids

Carotenoids are lipophilic, non-enzymatic antioxidant compounds that are highly abundant in plastids. They are recognized for their protective roles in safeguarding the photosynthetic apparatus under oxidative stress conditions. These pigmented compounds, synthesized by plants, contribute to various biological

processes, particularly the detoxification of singlet oxygen ($^1\text{O}_2$), thereby helping to prevent photo-oxidative damage.

In addition, carotenoids assist in maintaining the stability of light-harvesting complex proteins and thylakoid membrane structures, and they are essential components required for the formation and functionality of photosystem I (PSI) (Khan and Khan, 2017).

Flavonoids

Flavonoids also function as non-enzymatic antioxidants and are abundantly distributed in the aerial parts of plants, particularly in leaves, flowers, and pollen. In plant tissues, they are commonly stored in vacuoles, often in glycosylated forms (Ahmad et al., 2019). These compounds play diverse and essential roles in plant physiology, including flower and fruit pigmentation, as well as the regulation of overall plant productivity and performance.

Under oxidative stress conditions, flavonoids act as secondary scavengers of reactive oxygen species (ROS), thereby helping to prevent damage to the photosynthetic apparatus caused by excess excitation energy. Their strong antioxidant capacity is largely attributed to their structural properties, including radical accessibility and favorable redox potential, which enhance their ability to neutralize reactive species (Khan & Khan, 2017).

In addition, flavonoids are capable of detoxifying singlet oxygen ($^1\text{O}_2$) and protecting the outer structures of chloroplast membranes. They also inhibit lipid peroxidation and suppress lipoxygenase activity, thereby reducing membrane damage under stress conditions. As secondary metabolites, flavonoids have been reported to exhibit strong antioxidant efficiency, in some cases outperforming well-known antioxidants such as α -tocopherol and ascorbic acid (Ahmad et al., 2019).

Tocopherols

α -Tocopherol is a lipid-soluble antioxidant that serves a dual function by enhancing membrane stability and protecting cells from oxidative damage through the scavenging of reactive oxygen species (ROS) and lipid-derived free radicals (Ashraf et al., 2019). Among the four major tocopherol forms (α , β , γ , and δ), α -tocopherol is recognized as the most potent antioxidant. Tocopherols are commonly found in chlorophyll-rich green tissues of photosynthetic organisms, where they participate in cellular antioxidant defense mechanisms (Sadiq et al., 2019).

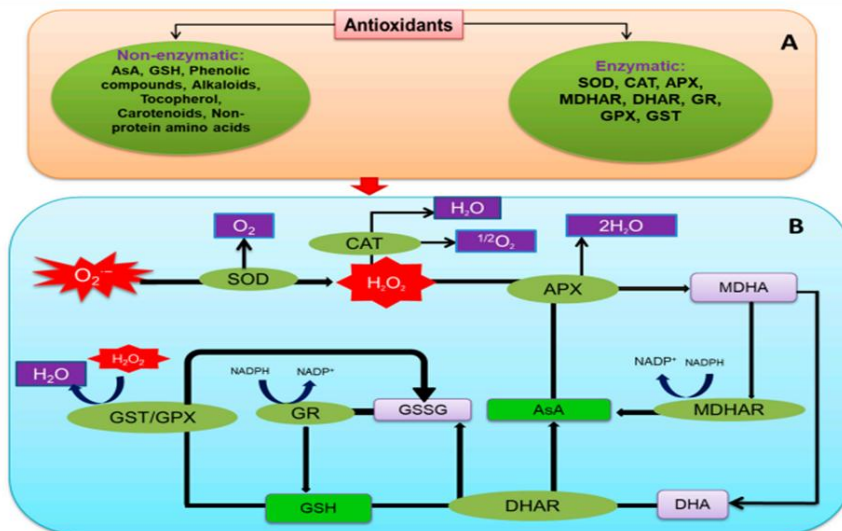


Figure 3. Overview of antioxidant defense mechanisms in plants; (A) types of antioxidants and (B) mechanisms by which antioxidant enzymes and low-molecular-weight antioxidants detoxify ROS (Hasanuzzaman et al., 2020)

The Role of ROS in Cellular Signal Transduction

Plants are equipped with a wide range of adaptive strategies that enable them to withstand adverse environmental conditions. One of the primary mechanisms involves the initiation of systemic signaling from stress-exposed tissues to non-stressed regions, thereby activating and priming defense responses throughout the

organism (Tsaniklidis et al., 2020). This systemic communication enhances stress resilience by integrating multiple signaling components, including reactive oxygen species (ROS), which function as key signal transducers in stress adaptation pathways (Kumar et al., 2017).

In addition to their well-known role in inducing oxidative damage, ROS also serve as important signaling molecules that promote tolerance to unfavorable environmental conditions (Camejo et al., 2016). When antioxidant scavenging capacity becomes insufficient, ROS accumulation may lead to an oxidative burst within plant cells. Under such conditions, the activation and modulation of ROS-mediated signaling pathways can help mitigate the detrimental effects of stress and restore cellular balance.

Exposure to both biotic and abiotic stresses induces dynamic changes in ROS production within cellular organelles, thereby triggering complex signal transduction networks that regulate homeostasis and stress responses (Saini et al., 2018). These networks involve tightly controlled feedback and feedforward loops that contribute to stress tolerance by maintaining ROS equilibrium and coordinating defense responses (Kumar et al., 2017).

Systemic ROS signaling has been described as a wave-like propagation that spreads from one cell to adjacent cells, enabling rapid long-distance communication within plant tissues (Mittler et al., 2011). This spatiotemporal signaling system enhances stress adaptation by coordinating responses across different plant regions. In addition to ROS, plants also utilize phytohormones and amino acids as signaling molecules to convey stress status and regulate adaptive responses (Kumar et al., 2017).

Strategies for Coping with Oxidative Stress

Enhancing the ability of plants to adapt to and tolerate abiotic stresses under changing climatic conditions represents a promising strategy for mitigating oxidative stress-induced damage. In this context, strengthening plant defense systems through the inhibition of pathways involved in excessive ROS production, improving antioxidant uptake, and modulating ROS-related signaling networks can significantly contribute to stress resilience. Such approaches may ultimately increase plant survival under adverse environmental scenarios.

Preconditioning plants to non-lethal stress and the application of molecular priming strategies using various elicitors, such as micronutrients (e.g., β -sitosterol) and osmolytes (e.g., GABA), have been shown to enhance defense capacity and reduce oxidative stress levels (Kerchev et al., 2020; Semwal & Khanna-Chopra, 2020). Molecular priming is therefore considered an effective tool for improving plant tolerance to abiotic stress by inducing a faster and stronger defense response upon subsequent stress exposure.

The integration of molecular priming approaches with systems biology frameworks further enhances their potential by enabling a deeper understanding of complex plant defense and tolerance mechanisms at the molecular level (Cramer et al., 2011). Systems biology focuses on the holistic interpretation of biological functions through multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics. These approaches facilitate the discovery of novel genes, RNAs, proteins, and metabolites, as well as the elucidation of their regulatory roles and intracellular interactions (Cramer et al., 2011; Liu et al., 2013).

Conclusion

Throughout their life cycle, plants are exposed to numerous environmental stress factors, particularly salinity, drought, extreme temperatures, heavy metal accumulation, UV-B radiation, pesticide exposure, and pathogen attacks. In response to these stress conditions, plants exhibit adaptations at molecular, biochemical, and physiological levels, primarily through antioxidant defense systems. Although reactive oxygen species (ROS) are toxic at high concentrations, they also function as key signaling molecules involved in stress perception, signal transduction, and the regulation of adaptive responses. Therefore, plants have evolved a highly coordinated antioxidant defense system to maintain ROS homeostasis.

Major enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), form the primary defense system by directly removing reactive oxygen species (ROS). Non-enzymatic, low-molecular-weight antioxidants, including ascorbate, glutathione, and flavonoids, provide additional protection through their radical-scavenging capacity. ROS production and accumulation in cellular organelles act as signaling molecules at low concentrations, whereas at high concentrations they function as toxic agents that cause oxidative damage. This dual function of ROS demonstrates how critical the precise regulation of redox balance (homeostasis) is in plants and highlights the essential role of the antioxidant defense system in tolerance and adaptation to environmental stress conditions.

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CHAPTER 15

CHRYSOPERLA SPECIES AS BIOLOGICAL CONTROL AGENTS IN SUSTAINABLE AGRICULTURE: BIOLOGY, ECOLOGY, AND APPLICATION POTENTIAL

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Introduction

Pests, diseases, and weed problems encountered in agriculture cause both quality and yield losses in cultivated plants; in addition to economic losses, this situation also poses a threat to food security (Kılınçer et al., 2010). Although chemical control methods have long been preferred to prevent these losses, such practices have, over time, led to environmental pollution, harm to non-target organisms, threats to human and animal health, and the development of resistance to pesticides (Özaktan et al., 2010). Furthermore, the increasing consumer demand for natural and residue-free products has intensified interest in alternative control methods.

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In this context, biological control stands out as an environmentally friendly and economically viable pest management strategy that is compatible with sustainable agricultural practices in the long term. Biological control agents, including parasitoids, predators, and entomopathogens, have the potential to suppress pest populations without disrupting the natural balance.

In particular, predatory species belonging to the family Chrysopidae have become one of the key components of biological control programs due to their broad prey spectrum, high adaptability, and compatibility with integrated pest management strategies. The Chrysopidae family is a cosmopolitan and species-rich group within the order Neuroptera, comprising more than 1,400 species and subspecies distributed across 82 genera (Lai et al., 2024; Lai & Liu, 2020). In Türkiye, approximately 50 species of the family Chrysopidae have been reported (Arı & Kıyak, 2000; Bozdoğan & Bahadıroğlu, 2015). Members of the Chrysopidae family are commonly known as green lacewings. Although not all adult Chrysopidae species are predatory, their larvae are predaceous. Non-predatory adults feed on plant-derived resources such as nectar and pollen, as well as on honeydew secreted by certain insects (e.g., aphids) (Alghamdi, 2015; Lai & Liu, 2020; Pappas et al., 2011).

One of the most important genera within the family Chrysopidae in biological control programs is *Chrysoperla* Steinmann (Henry et al., 2001). This genus, which is particularly used in the management of agricultural pests, has produced successful results in many countries with species such as *C. carnea*, and it is widely preferred. The genus *Chrysoperla* comprises 36 different species (Abdolahadi et al., 2022; Morales & Freitas, 2010; Morales-Correa e Castro & Barbosa, 2017).

Chrysoperla spp. are widely used as an environmentally friendly alternative to chemical pesticides in agriculture due to their host range, geographical distribution, and resistance to certain

pesticides (Abdolahadi et al., 2022). These natural enemies play a crucial role in maintaining ecosystem balance by standing out for their ability to suppress pest populations. For instance, the larvae of green lacewings, which feed on numerous economically important pests such as aphids, scale insects, leafhoppers, whiteflies, mealybugs, mites, thrips, and the eggs and early larval stages of Lepidoptera species, provide an effective solution for protecting crop health (Chen et al., 2006; Lai & Liu, 2020; Martins et al., 2019; Mirmoayedi et al., 2014; Pappas et al., 2011; Tian et al., 2023).

Many species within this genus have long been used as important biological control agents against agricultural pests due to their high predation capacity, broad adaptability, strong reproductive potential, and ease of mass rearing. In addition, their suitability for commercial production and distribution makes them an ideal option for promoting sustainable agricultural practices (Lai et al., 2024; Lai & Liu, 2020; Pappas et al., 2011). Owing to the significant value of green lacewings in biological control, research focusing on their basic biology, behavior, and application techniques has gained considerable attention. In recent years, extensive studies have been conducted at both local and international levels on their biological characteristics, predatory efficiency, artificial rearing, and release strategies under laboratory and field conditions (Lai & Liu, 2020).

The main objective of this section is to compile up-to-date information on the use of *Chrysoperla* species as biological control agents in agricultural production and to facilitate their identification. The review covers their biology, ecology, predatory behavior, impact on target pest species, mass-rearing techniques, and potential in integrated pest management (IPM). In this respect, the study aims to serve as a guiding and educational resource for both researchers and agricultural practitioners.

Biological Characteristics of *Chrysoperla* spp.

Species belonging to the Chrysopidae family exhibit similar morphology, with membranous and two-paired wings. A characteristic feature that distinguishes this family from other insect orders is the habit of folding their wings roof-like over the body during rest. The wings are relatively primitive in structure and are densely reticulated with numerous cross-veins (Toroglu et al., 2012).

The larvae are approximately 2 mm in length and vary in color from brown to greyish tones. Their sickle-shaped, sharply pointed mandibles are connected to salivary glands and contain toxic compounds; these structures allow them to seize prey, immobilize it, and subsequently consume its internal body fluids (Toroglu et al., 2012).

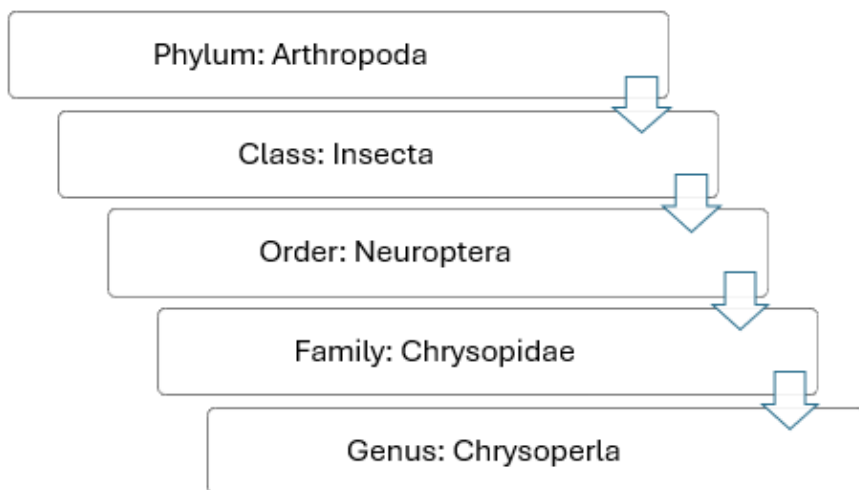


Figure 1. Taxonomic classification scheme of the genus *Chrysoperla*.

The life cycle of *Chrysoperla* species consists of four main stages: egg, larva, pupa, and adult. The larval stage includes three instars. Among these, the highest feeding activity is observed during the third instar. Each larval stage is characterized by a progressively

larger body size and increasingly efficient prey-capture ability compared to the previous one.

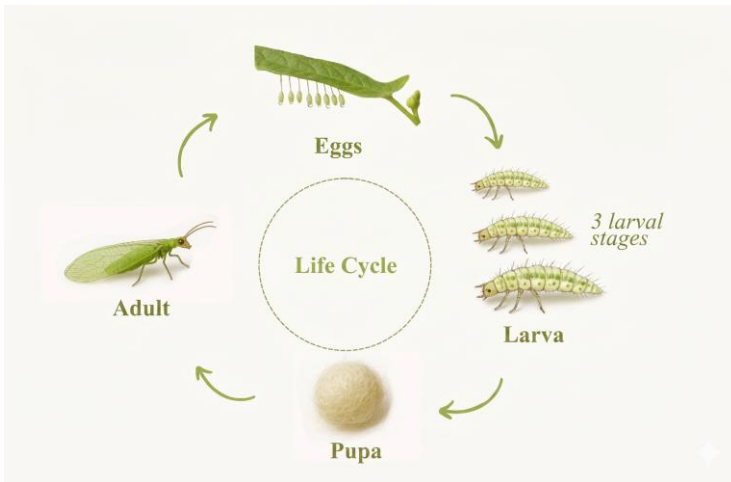


Figure 2. Life cycle of *Chrysoperla* species.

Female *Chrysoperla* individuals typically lay their eggs singly or in small clusters on leaf surfaces or young shoots where prey density is high (Pappas et al., 2011). The eggs are characteristically deposited on a slender stalk. This stalk-like structure is thought to protect the eggs and newly hatched larvae from predators such as ladybird beetles, thereby increasing their survival rate (Lai & Liu, 2020).

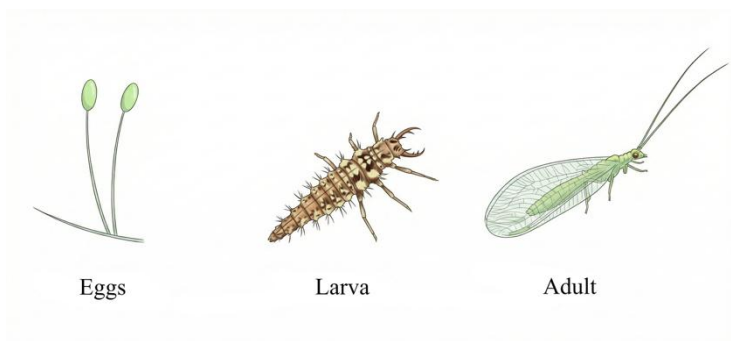


Figure 3. Egg, larval, and adult stages of *Chrysoperla* species.

Predatory Potential of *Chrysoperla* spp.

Chrysoperla spp. have long been used as effective natural enemies in biological control of agricultural pests due to their high predation capacity, broad adaptability, strong reproductive potential, and ease of mass rearing. These species are widely preferred in agriculture as an environmentally friendly alternative to chemical pesticides, owing to their diverse host range, wide geographical distribution, and resistance to certain insecticides (Abdolahadi et al., 2022).

While green lacewing larvae are predatory, adult individuals are not. Larvae feed on a wide range of economically important pests, including aphids, scale insects, leafhoppers, whiteflies, mealybugs, mites, thrips, and the eggs and early larval stages of Lepidoptera species. In contrast, non-predatory adults primarily feed on plant-derived resources such as nectar, pollen, and honeydew secreted by certain insects (Alghamdi, 2015; Lai & Liu, 2020; Pappas et al., 2011).

Chrysoperla species, particularly *C. carnea*, are widely used and have produced successful outcomes in pest management programs across many countries. Numerous studies have demonstrated the high predatory efficiency of *C. carnea* against various pest species. Laboratory experiments have shown that third-instar larvae exhibit the highest predation rates against both eggs and first-instar larvae of *Helicoverpa armigera* (Hassanpour et al., 2011). Similarly, feeding behavior varies significantly depending on prey type; under laboratory conditions, larvae consumed an average of 633 individuals of *Sitotroga cerealella*, followed by 444 individuals of *Ephestia kuehniella* and 367 individuals of *Aphis gossypii*, indicating that feeding efficiency varies with prey quality and species (Saleh et al., 2017).

The biological performance of *C. carnea* also varies depending on host species. In a study by Sattar et al. (2011), larvae were reared on *Sitotroga cerealella*, *Aphis gossypii*, *Phenacoccus solenopsis*, *Pectinophora gossypiella*, and *Helicoverpa armigera*. The longest larval developmental period was observed on *S. cerealella*, while this host also resulted in the highest survival rate and the greatest fecundity in adult females. These findings indicate that *C. carnea* exhibits strong developmental and reproductive performance on certain prey such as *S. cerealella* and *A. gossypii*.

The third larval instar is particularly noteworthy in terms of predatory efficiency. In studies conducted on *A. gossypii*, maximum aphid consumption occurred during the third larval stage, which also exhibited the longest survival under prey-free conditions, highlighting its critical role in both feeding activity and survival (Yüksel & Göçmen, 1992).

Similarly, studies on *Phenacoccus solenopsis* showed that all larval stages preferred first-instar nymphs; however, third-instar larvae consumed all nymphal stages at significantly higher rates, making them the most effective predators (Rashid et al., 2012). These results emphasize differences in predatory efficiency among larval instars and highlight the importance of third-instar larvae in biological control.

Experiments on two-spotted spider mites (*Tetranychus urticae*) demonstrated that first- and second-instar larvae exhibited a Type II functional response, while third-instar larvae showed a Type III functional response. Predation capacity increased with larval development, reaching a maximum of approximately 188 prey individuals in the third instar (Hassanpour et al., 2009). These findings clearly indicate ontogenetic variation in predatory behavior.

Furthermore, studies on *Pieris brassicae* revealed that third-instar larvae possess the highest predation capacity, with a strong

preference for caterpillars. Huang and Enkegaard (2010) reported that in the presence of alternative prey such as aphids, second-instar larvae reduced their attacks on eggs and caterpillars, whereas third-instar larvae did not show this behavioral shift, indicating a more consistent and effective predatory strategy.

Overall, *C. carnea* larvae demonstrate high predation capacity against a wide range of agricultural pests. Gautam and Tesfaye (2002) reported that a single larva can consume approximately 216–950 aphids, 510.8 whitefly pupae, 160–200 eggs of *H. armigera*, 147 *Drosophila melanogaster* larvae, and 350 eggs of *Corcyra cephalonica* during its development. These data clearly highlight the potential of *C. carnea* in integrated pest management (IPM) programs.

In conclusion, comprehensive studies indicate that *Chrysoperla carnea* larvae are highly effective predators against various pest species, with predation capacity peaking during the third larval instar. This developmental stage is considered critical both in terms of feeding efficiency and survival, and its strategic utilization may enhance the effectiveness of biological control programs. Accordingly, the third larval stage of *C. carnea* represents a key component in its potential application for sustainable pest management in agricultural systems.

Use in Biological Control and Mass Rearing

Chrysoperla spp. are mass-reared in many countries for biological control of agricultural pests. The fact that adults are not predatory greatly facilitates their large-scale production under laboratory conditions. Non-predatory adults are typically fed a protein-rich liquid diet composed of protein hydrolysate, sucrose, and water. However, Pappas et al. (2011) reported that mass rearing of predatory adults is operationally more complex and cost-intensive.

In commercial production of green lacewings (*Chrysoperla* spp.), eggs of moth species belonging to the genera *Sitotroga*, *Ephestia* (Anagasta), and *Corcyra* are commonly used as the primary food source for larvae, which represents a critical stage in their production cycle (Nordlund et al., 2001; Pappas et al., 2011). Although artificial diets have been developed to replace insect eggs, production processes still largely rely on labor-intensive practices (Nordlund et al., 2001). Nevertheless, these eggs remain a nutritionally rich and relatively cost-effective food source compared with many artificial diets (Pappas et al., 2011).

Nordlund et al. (2001) reported that in North America, there were 65, 20, and 54 suppliers producing *Chrysoperla carnea* (Stephens), *C. comanche* (Banks), and *C. rufilabris* (Burmeister), respectively. The same study also noted that *C. carnea* is widely used in Europe, while *C. sinica* (Tjeder) is commonly utilized in China.

As of 2025, mass production of *Chrysoperla* species for biological control applications is widely practiced in many countries worldwide. Leading companies in this field include Koppert Biological Systems (Netherlands), BioBee (Israel), Bioline AgroSciences (United Kingdom and France), ARBICO Organics (USA), Biological Services (Australia), Applied Bio-Nomics Ltd. (Canada), and Plantsman Group (United Kingdom). In addition, according to SENASICA (2015), at least 35 companies in Mexico are involved in the production of *Chrysoperla* species, while in countries such as Pakistan, production and field applications are conducted by both public institutions and private sector organizations.

This extensive production network is directly related to the effectiveness of *Chrysoperla* species in biological control, their environmentally friendly nature, and their relatively ease of rearing under laboratory conditions. Green lacewings (*Chrysoperla* spp.) are widely recognized as effective and generalist predators used in

integrated pest management (IPM) strategies. Their broad prey spectrum, high searching efficiency, and tolerance to environmental stress factors make them indispensable components of IPM programs. In addition, their potential to develop a certain level of resistance to chemical pesticides enables the integrated use of chemical and biological control methods (Dey & De, 2018; Mansoor & Shad, 2020).

A study by Khan and Shad (2025) highlighted the insecticide resistance potential of *C. carnea*, its relatively easy mass production, and its high adaptability to environmental variability, further emphasizing its strategic importance in IPM systems. Such findings strengthen the scientific basis for the use of *Chrysoperla* species as sustainable, environmentally friendly, and effective biological control agents.

Chrysoperla species are commercially available natural enemies widely used across home gardens, row crops, orchards, and greenhouse systems (Fathipour & Maleknia, 2016). This broad utilization is directly associated with their adaptability and effectiveness across diverse ecosystems. They have been successfully applied in various agricultural production systems against a wide range of pests. In particular, they are highly effective against aphids and have been released in crops such as pepper, eggplant, pea, potato, and cotton for aphid suppression. They have also been reported to be effective against important pests such as *Leptinotarsa decemlineata* (Colorado potato beetle) in eggplant, *Panonychus ulmi* (European red mite) in apple orchards, and *Heliothis virescens* (tobacco budworm) in cotton fields.

In greenhouse systems, species such as *C. carnea*, *Chrysopa septempunctata*, *Chrysopa formosa*, and *C. perla* have been successfully used for aphid control in crops including pepper, cucumber, eggplant, and lettuce. In North American applications, *C. carnea* is widely used against aphids, mealybugs, and cicadellids,

while *C. rufilabris* is preferred for whiteflies, aphids, and cicadellids (Pappas et al., 2011). Additionally, *Chrysoperla externa* has been reported to be used against noctuid larvae, while *C. plorabunda* is particularly effective against aphids (Pappas et al., 2011).

This broad application range demonstrates the strong adaptability and effectiveness of *Chrysoperla* species across different agricultural systems and pest groups. Future research is expected to further expand and optimize their potential in biological control programs.

Interactions of *Chrysoperla* Species with Other Natural Enemies

The effectiveness of biological control programs depends not only on the impact of released natural enemies on target pest populations, but also on their interactions with other natural enemies present in agroecosystems. *Chrysoperla* species, particularly *C. carnea*, exhibit various interactions with other predators and parasitoids. These interactions may occur in the form of competition, intra- and interspecific predation, and indirect ecological effects.

Rosenheim et al. (1999) investigated the interactions of *Chrysoperla carnea* with other generalist predators in cotton agroecosystems. The authors emphasized that traditional biological control theory is based on a three-trophic-level model (plants, herbivores, and predators). However, in real ecosystems, predators interact not only through shared prey competition but also through intraguild predation. In cotton agroecosystems, it was found that Hemipteran predators caused up to 90% predation on *C. carnea* larvae, which negatively affected the biological control of aphid populations (Rosenheim et al., 1999).

Intraspecific cannibalism is another important interaction observed in *Chrysoperla* species. Pappas et al. (2011) reported that under conditions of high population density and food scarcity, *Chrysoperla* larvae may consume eggs and early instar larvae of

their own species. This behavior is particularly important for determining optimal release rates in mass release programs.

Interactions of *Chrysoperla* species with other natural enemies have also been studied within the context of tritrophic relationships. Farrokhi et al. (2017) demonstrated that the predator–prey relationship between *C. carnea* and *Myzus persicae* varies depending on the host plant species. These findings indicate that tritrophic interactions and life table analyses of natural enemies contribute significantly to the improvement of integrated pest management (IPM) strategies (Farrokhi et al., 2017).

Understanding the interactions of *Chrysoperla* species with other natural enemies is crucial for enhancing the efficiency of biological control programs. In particular, minimizing negative interactions such as intraguild predation and competition, while promoting synergistic effects, can significantly improve the success of biological control applications. Therefore, future research should focus on examining these interactions in greater detail under different agroecosystem conditions.

Conclusion

In the context of increasing importance of sustainable agricultural practices, biological control methods have emerged as a promising alternative to chemical pesticides. In this review, the use of species belonging to the genus *Chrysoperla* as biological control agents, their biological characteristics, predatory potential, and mass-rearing techniques have been comprehensively examined. The findings clearly demonstrate that *Chrysoperla* species, particularly *C. carnea*, are effective predators in the biological control of agricultural pests.

The morphological and biological characteristics of *Chrysoperla* species form the foundation of their success in biological control. In particular, the high predation capacity of larval

stages, their broad prey spectrum, and their ability to adapt to different ecological conditions make these species valuable components of integrated pest management (IPM) programs. Studies have shown that *Chrysoperla* larvae are effective predators of aphids, whiteflies, thrips, mites, and the eggs and early larval stages of various Lepidoptera species. Notably, third-instar larvae exhibit the highest performance in terms of both prey consumption and survival rate. This highlights the strategic importance of utilizing third-instar larvae in biological control applications.

The relatively easy mass production of *Chrysoperla* species is another key factor supporting their widespread use in biological control programs. The non-predatory nature of adults facilitates large-scale laboratory rearing and reduces production costs. Worldwide, numerous private companies and public institutions are engaged in the production and commercialization of *Chrysoperla* species. This extensive production network reflects the biological efficacy and economic value of these species in pest management.

However, several challenges must be addressed to ensure the effective use of *Chrysoperla* species in biological control. Factors such as release timing, release rates, target pest population density and phenology, climatic conditions, and agricultural practices can significantly influence their effectiveness. Therefore, further field studies are needed to optimize the performance of *Chrysoperla* species across different agroecosystems and against various pest species.

In addition, compatibility with chemical pesticides is an important consideration for the integration of *Chrysoperla* species into IPM programs. The potential development of resistance to certain pesticides may facilitate their integration with chemical control methods. However, susceptibility to pesticides may vary among species and even among populations within the same species. Thus, a better understanding of pesticide sensitivity and the careful

planning of pesticide applications are necessary to minimize negative impacts on their effectiveness.

Future research should focus on developing new release techniques, habitat management strategies, and genetic improvement approaches to enhance the efficacy of *Chrysoperla* species in biological control. In particular, genetic studies aimed at improving adaptation to environmental conditions and increasing predation efficiency may further enhance their potential. Moreover, interactions between *Chrysoperla* species and other natural enemies, as well as their implications for biological control programs, should be investigated in greater detail.

In conclusion, this review highlights the significant role and potential of *Chrysoperla* species in biological control. A better understanding of their biology, ecology, and predatory behavior will support their more effective use in pest management programs and contribute to the development of environmentally friendly pest control strategies in agricultural production. The integration of natural enemies such as *Chrysoperla* species into IPM programs is essential for reducing dependence on chemical pesticides and promoting sustainable agriculture. Strengthening the role of *Chrysoperla* species in biological control will therefore make important contributions to both economically and ecologically sustainable agricultural systems.

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