

PLANT PHYSIOLOGY: FROM HISTORICAL ROOTS TO FUTURE FRONTIERS

Editor:
Ergun Kaya

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The background of the cover is a photograph of a pink flower with a red center, likely a coneflower. A small bee is perched on one of the petals. The background is blurred, showing other flowers and greenery. The top of the cover has a green geometric shape containing the title.

Current and Future Developments in Physiology

(Volume 2)

Plant Physiology: From Historical Roots to Future Frontiers

Edited by

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(Volume 2)

Plant Physiology: From Historical Roots to Future Frontiers

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FOREWORD

Current and Future Developments in Physiology (Volume 2) is a book that is published for the benefit of botany. The people spearheading the initial steps of this service hope that this book will serve as a valuable resource for all plant physiologists in publishing important, fundamental discoveries that further our understanding of plant growth, development, and metabolism. The editor and authors of the chapters see their work as providing committed support to the study of plant physiology as a whole.

With the rapid advancement of technology, science has begun to occupy an increasingly larger place in the lives of humanity. Research in plant physiology is increasingly evolving into the fields of artificial intelligence and bioinformatics, which include computerized applications. Concurrently, it constantly investigates in more depth the problems of developmental metabolism under the leadership of physiologists well-trained in molecular approaches, biophysics, and biochemistry methods. In this context, this book combines previous knowledge in the field of basic plant physiology with artificial intelligence and bioinformatics, including biotechnology and molecular biology approaches.

Research is uncovering actual issues that are pressing the greatest intellectual pursuits of humanity to address. Therefore, this book is available for all plant sciences, where physiological approaches must be employed to solve encountered difficulties in order to improve this major field of research overall. In addition to serving as a method for bringing all plant physiologists together into a cohesive, effective working group, my hope is that it will serve as a resource for plant physiologists across all fields of study by offering a central setting in which we can collaborate in the advancement of plant physiology without interfering with other groups' scheduled activities.

I think that this book, which consists of eleven chapters, each containing useful information, will be an even more useful resource with the support and constructive criticism of basic science plant physiologists and applied physiologists from all nations. I would like to express my endless thanks to the editor, book authors, publication editors, graphic designers, and all those who contributed to the preparation and publication of the book. I hope you will read the book as a useful resource.

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PREFACE

Plant physiology is a science that studies the symptoms and causes of various vital events that occur throughout the life of plants. The vital events occurring in plants are the result of chemical and physical changes in the living matter of the cell. So, in more general terms, we can define the events that occur as a result of the physical and chemical changes that occur in living things as physiological. Plant physiology tries to answer the question of how and why these physiological events occur in plants, and thus plant physiology reveals the laws and principles in force for physiological events. While presenting these laws and principles, the laws of physics and chemistry are undoubtedly used to a large extent. This reveals that physiology has a close relationship with physics and chemistry. It should also be noted that plant physiology has a special importance in biology because it is a science based on quantitative results, just like chemistry and physical sciences. Because plant physiologists must not only provide descriptive explanations but also explain events with quantitative values.

This book of eleven chapters on classical plant physiology and basic mechanisms aims to present to the reader, by combining many approaches based on the molecular basis of the development of plant physiology, the elucidation of basic metabolic pathways, and the events taking place at the cellular level, starting from the history of plant physiology to current molecular approaches and artificial intelligence-supported applications.

I would like to thank all the book authors who made valuable contributions to the preparation of this book, my advisors Prof. Dr. Fusün GÜMÜŞEL, Prof. Dr. Yelda ÖZDEN ÇİFTÇİ, Dr. Fernanda VIDIGAL DUERTA SOUZA and Dr. Dave ELLIS, who helped me excel in this field and provide useful products, and our families who always supported me.

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*To the world you are a mother, but to your children you are the world,
Dedicated to my mother and all mothers...*

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CHAPTER 1

Historical Development of Plant Physiology

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Abstract: Although the basis of plant science is identified with the history of humanity, studies in the field of plant physiology based on both the development process of science and technological developments date back to the very recent past. In 1727, English physiologist, inventor, and chemist Stephen Hales published a book called 'Vegetable Statick' and in his book, Hales explained how water is mobilized in plants and laid the foundations of plant physiology. Since then, great developments in technology and biotechnology have allowed plant physiology to grow in a logarithmic manner. Today, many metabolisms have been enlightened both at the cellular level and at the tissue and organ level, and new studies are being added to these studies every day. In addition to the significant advances brought about by technological advancement, research in the fields of nutrition, plant chemistry, particularly in the agricultural sector, and genetics and molecular biology, though often fraught with ethical issues, has produced some truly groundbreaking discoveries. Within this framework, the goal of this chapter is to elucidate the features of the development processes by examining the history of plant biotechnology development, how technological advancements have accelerated this process, and what key studies were conducted during these phases.

Keywords: Biotechnology, Cellular events, Plant metabolism, Plant physiology.

INTRODUCTION

Plant physiology investigates the biological events that occur during the life process of a plant, from the beginning as a seed to the stage of producing seeds again. Plant functions are basically based on the principles of physics and chemistry. Plant physiology is studied with the application of modern physics and chemistry techniques to the plants studied, so research in plant physiology also deepens with the advancements in these branches of science [1, 2].

The development of tissues in plants is closely related to the environment in which the plant is located and the physiological events that occur accordingly.

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Light, humidity, temperature, water, and gravity are important environmental factors affecting plant growth. Structure and function are closely related; that is, living things come into question because of the coexistence of genes, enzymes, other molecules, organelles, cells, tissues, and organs. For this reason, studies on plant physiology are closely related to plant anatomy, cell biology, and structural and functional chemistry [3, 4].

The target principle of plant physiology is how plants reproduce, grow, and develop. From ancient times to the present, when people started collecting seeds to grow nutritional plants, they saw that plants needed optimum environmental conditions such as sunlight, warmth, and moist soil and that the best-quality seeds produced the most productive plants [5]. They did, however, observe the advantages of practices like fertilization, irrigation, and hoeing. Agricultural activities, which spanned a very long time, enabled the development of new varieties and the cultivation of various species (Fig. 1). The information obtained from basic plant growth and morphological analyses, which are among all these preliminary study activities, laid the foundations of plant physiology [5, 6].



Fig. (1). Medicinal-aromatic plant species cultivated in the collection garden of Muğla Metropolitan Municipality, Department of Agricultural Services, Garden for Local Seed Center. **a.** *Echinacea purpurea* (L.) Moench; **b.** *Salvia fruticosa* Mill.

Early Experiments on Growth and Development

The first physiological approach to growth was directed at answering the question of where a plant gets the components necessary for its growth. Jean Baptista Van Helmont [7], who lived in the early 1600s, made a suggestion for this approach. According to Helmont, a Belgian doctor, the only source for the growth and development of a plant was water. The researcher irrigated the willow sapling he planted in a pot with only rainwater and grew a huge tree on a very small piece of soil. The researcher knew about CO₂ at the time but never anticipated that it could be one of the key growth drivers [7]. Some studies that have provided significant developments in plant physiology are listed chronologically in Table 1, and are

briefly summarized. In the 1700s, Antoine Lavoisier discovered that the matter resulting from organic synthesis was composed of oxygen and carbon on a large scale [8].

Table 1. Some of the studies that pioneered the development of plant physiology [7 - 42].

Researcher	The Purpose of the Work	Date
Jean Baptista Van Helmont	Finding the answer to the question of where the plant gets the components necessary for its growth on <i>Salix</i> sp.	1600s
Antoine Lavoisier	He found that the substance formed as a result of organic synthesis consists of oxygen and carbon on a large scale.	1700s
Stephen Hales	He published his work “Vegetative Statics” as a result of his studies on transpiration, plant growth and gas exchange in plants.	1727
Joseph Priestley	He showed that plant leaves take in carbon dioxide in light and emit an equivalent amount of oxygen.	1770
Jan Ingenhousz	He repeated Priestley's experiments and investigated the effect of sunlight on living things.	1778
Jean Senebier	He carried out studies on the effect of sunlight on nature and photosynthesis.	1790
Nicholas de Saussure	He noted that water is involved in the photosynthesis process. Additionally, his findings on plant physiology have been reported in detail in many sources.	1807
Justus von Liebig	He introduced the “Law of the Minimum”, which is the view that the growth and development of plants is limited by the least amount of nutrients in the soil.	1830s
Julius Robert von Mayer	observed that the process converts light energy into the chemical energy of organic carbon	1842
Julius Sachs	He carried out the first studies on the uptake of nutrients and mineral substances from the soil by plants.	1865
Henry Dixer and John Joly	They studied the mechanism by which water and mineral substances are drawn up from the roots of plants with the help of open vessels of the xylem and tracheids.	1895
Gottlieb Haberlandt	He cultured the isolated plant cells in a simple nutrient solution, and this attempt was important in that it gave an idea about the potential of the cell and demonstrated a new technology as plant tissue culture.	1902
Wightman W. Garner and Harry A. Allard	They discovered photoperiodism, the regulation of flowering according to day length.	1920
Cornelis Bernardus van Niel	He used radioactive water to show that water, not carbon dioxide, was the source of the oxygen released during photosynthesis	1929
Erwin Bünning	He found that sleep movements, such as the drooping of bean leaves in the evening, are controlled not by the onset of darkness but by a biological “clock,” a circadian rhythm.	1930s

Stress Physiology and Current Approaches

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Abstract: Plants often encounter environmental stressors in both wild and cultivated environments. Certain environmental stressors, like air temperature, only last a few minutes, but others, like soil water content, might persist for several days. Stress might last for months if there is a mineral shortage in the soil. This chapter gives an overview of the ways that soil, climate, and stress affect the spread of different plant species. Thus, it is crucial for agriculture and the environment to comprehend the physiological mechanisms that underlie plants' methods of adaptation and acclimatization to environmental challenges. A common definition of stress is an outside influence that negatively impacts plants. Stress tolerance and the concept of stress are closely related. The capacity of a plant to withstand adverse environmental conditions is known as stress tolerance. One plant may not find stress in the same environment as another. Based on the fundamental ideas of stress physiology in plants, this chapter seeks to provide a modern and fundamental explanation of the metabolic processes that occur in cells.

Keywords: Antioxidant mechanism, Heat stress, Salt stress, Toxicity, Water stress.

INTRODUCTION

Living things, by their nature, are in constant contact with the external environment. If inappropriate conditions occur in their environment, they are exposed to stress conditions due to lack of adaptation. When environmental conditions change so much that they negatively affect the normal growth and development of a plant, the situation that occurs in the plant is called stress. In other words, it is defined as external factors that have negative effects on the plant. In many cases, stress is a concept that needs to be explained by relating it to the survival of the plant, its ability to produce products, biomass accumulation and assimilation [1, 2].

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As a matter of fact, abiotic stresses such as drought, salinity, extreme temperatures, chemical toxicity, and oxidative stress are serious threats that disrupt agricultural activities and deteriorate the environment. For example, abiotic stress is the primary cause of crop yield loss worldwide, greatly reducing average crop yields in the most productive crop plants. Accumulated data on how plants respond to biotic and abiotic stress and how stress affects the developmental processes in the plant life cycle have enabled the development of new approaches on the subject [3, 4].

Plants encounter many stress factors during their lives. According to Levitt, stress factors are divided into two: biotic and physicochemical [5]. Biotic factors include stress factors caused by the infection of microorganisms (fungi, bacteria, and viruses) and attacks by harmful animals. Abiotic factors are environmental factors such as water, temperature, radiation, chemicals, magnetic and electrical fields [6]. Plants, which do not have the option of avoiding the stressor by moving away from it due to their sessile nature, are directly exposed to stress, unlike animals. This direct effect negatively affects growth and development and causes plant organs to lose their vitality [7, 8].

Damage caused by stress factors varies depending on the plant type, tolerance, and adaptation ability [9, 10]. Considering that plants encounter many stress factors in nature throughout their lives, it is very important to elucidate stress-related mechanisms and develop tolerant species and varieties. For this purpose, in this chapter, the molecular and biochemical events that occur in plants under stress conditions will be discussed and the responses to stress will be tried to be explained [7, 11].

RESISTANCE TO WATER SHORTAGE AND DROUGHT

Drought resistance mechanisms are divided into several types. First, postponement of drying (the ability to retain water in the tissue) and tolerance to drying. These two are sometimes referred to as drought tolerance at high and low water potentials, respectively. A third mechanism is escape from drought. This mechanism involves completing the life cycle during the rainy season, before drought occurs. There are two categories of people who delay drying: those who do not waste water and those who do. Individuals who do not waste water use it in moderation. In order to use it later in life, these plants store part of the water in the soil. Individuals that wastewater consume a lot of water. The mesquite tree (*Prosopis glandulosa* Torr.) is an example of a water waster. This plant, whose roots can reach very deep, has destroyed the semi-arid grasslands in the southwestern United States [12]. Since it uses excessive water, it prevents grasses

of agricultural value from settling in that area. A plant with a high ability to gain and use water will be more resistant to drought [12, 13].

Some plants have adaptations such as the C₄ and CAM photosynthesis pathways (Fig. 1). These adaptations allow plants to use more water. Additionally, plants have acclimation mechanisms that are activated to respond to water stress. Water scarcity is defined as any water content of a tissue or cell below the highest water content in the plant. Water stress has some effects on growth: it is the limitation of leaf expansion (*i*), water shortage stimulates leaf abscission (*ii*), during water scarcity, roots grow towards moist regions deep in the soil (*iii*), stomata close in response to abscisic acid during water scarcity (*iv*), water shortage limits photosynthesis (*v*), osmotic stress promotes *Crassulacean Acid Metabolism* (CAM) in some plants (*vi*), and leads to changes in gene expression (*vii*) [14, 15].

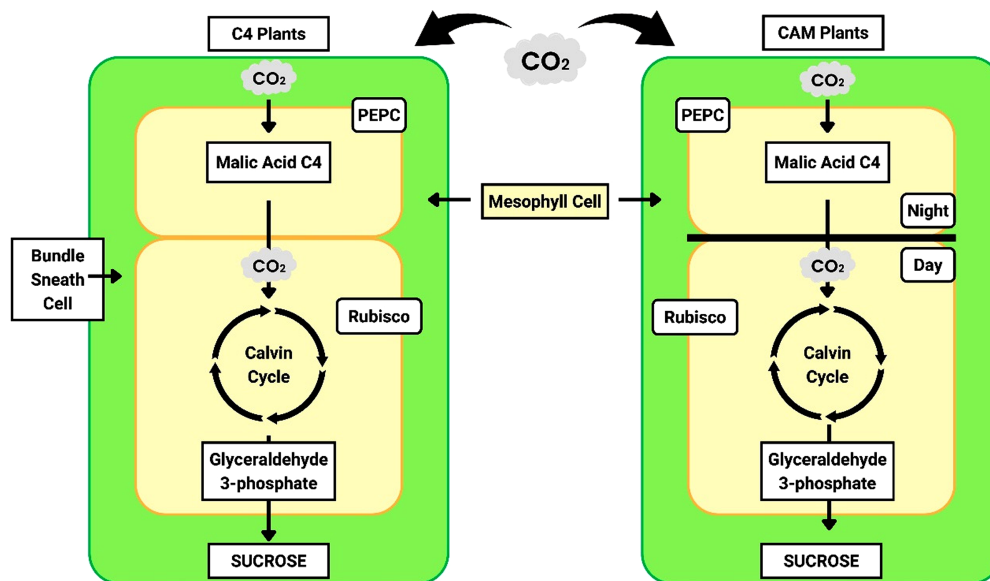


Fig. (1). Types of photosynthesis in C₄ and CAM [18].

Water Scarcity Limits the Leaf Expansion

As the water content of the plant decreases, the cells shrink and the cell walls become looser. This decrease in cell volume causes the turgor pressure in the cells and subsequently the solute concentration to decrease. As the area it covers decreases, the plasma membrane thickens and the pressure on it increases. Decreased turgor is the first and most important biophysical effect of water stress. Inhibition of cell expansion in the early stages of water scarcity slows leaf expansion. As the leaf area decreases, water loss through transpiration decreases.

CHAPTER 3**New-Generation Plant Growth Regulators****Ergun Kaya^{1,*} and Damla Ekin Özkaya^{1,2}**¹ *Muğla Sıtkı Koçman University, Faculty of Science, Molecular Biology and Genetics Department, 48000, Menteşe, Muğla, Türkiye*² *Okan University, Vocational School of Health Services, Medical Laboratory Techniques Department, 34959, Tuzla, İstanbul, Türkiye*

Abstract: It is known that metabolic conditions such as differentiation, growth, flower and fruit formation, and development in plants are mostly organized by the plant growth regulators. These organic substances that can be made naturally in plants, control growth and other metabolic conditions related to it. They can be carried from where they occur to other parts of the plant. They can be efficient even at very small volumes and are called plant growth regulators. These are the most significant molecules affecting the subsequent plant growth and development and the internal formation of different metabolic reactions. Growth regulators were initially used only for germination of seeds and rooting of cuttings. Later, it has also been used to increase yield, product quality, and the resistance of plants against pests and diseases in the period from seed to harvest. Plant growth regulators can contribute to increasing plant resistance against diseases by stimulating the plant defense system through various physiological or biochemical reactions that occur as a result of host-pathogen interaction.

Keywords: Absciscic acid auxin, Cytokinin, Ethylene, Gibberellic acid.

INTRODUCTION

The main internal factors that regulate the growth and development of the plant are chemicals. Plant growth regulating substances can be produced by plants or given externally to the plant in very small amounts. In addition, they are organic substances that can affect growth, development and other physiological events in the plant positively or negatively. Plants themselves produce these basic substances that they need for growth, development and change. These substances that are formed in the plant and regulate growth and development (physiological events) are called plant growth regulators (PGR) or phytohormones (plant hormones) [1 - 3].

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The importance of PGR was understood for the first time in the 1930s, and from this date on, their function in agricultural products began to be investigated. Danish botanist Went has experimentally demonstrated that a growth agent flows from these cut ends by fitting the cut coleoptile ends onto agar blocks. When this researcher cut the coleoptile apex, he saw that the growth rate of the coleoptile was greatly reduced. He observed that when he reattached the cut end to the top of the cut coleoptile, the growth of the coleoptile began again [4, 5]. Studies on plant physiology have revealed the roles of PGR in plant growth and development, and it has been understood that not only growth-promoting substances but also growth-inhibiting substances are synthesized in the plant over time [6 - 8]. Today, the effects of PGRs, which are widely used to affect the growth rate and development of the plant from germination to harvest and post-harvest storage, on yield are generally indirect [9, 10]. In the external application of these substances to plants, the selection of the appropriate chemical, the determination of the appropriate concentration, and the application time are very important for the desired effect [11, 12].

The main purposes of using PGRs in agriculture can be listed as follows: To ensure propagation by cuttings, to increase the germination power of seeds, to encourage or delay flowering, to increase cold resistance, to increase seed formation in fruits, to increase fruit size, to extend the storage time of fruit, to increase resistance of plants to diseases and pests, to control weeds, to prevent lodging in cotton and cereals [1, 7, 13, 14], to prevent fruit shedding before harvest, to enable all plants to mature at the same time to facilitate machine harvesting, to reduce labor at harvest, to prevent dormancy by accelerating ripening, to break dormancy, to encourage root-shoot and tuber formation especially in tissue culture studies [15 - 17]. The nature, formation and effects of growth regulators found naturally in plants are given in Table 1.

Table 1. The effects of the growth regulators naturally found in plants [1, 10, 13, 18].

Plant Growth Regulator	The Part of the Plant where the Growth Regulator is Synthesized	Effect of Growth Regulator on the Plant
Auxins	Synthesized in leaf primordia, young leaves, and developing seeds.	Promote stem elongation, root growth, cell differentiation, and branching and regulate phototropism and fruit development.
Cytokinins	Synthesized in root tips.	Promote root growth and differentiation, cell division and growth, and seed germination.
Gibberellins	Synthesized in young tissues of shoots and developing seeds.	Promote seed and bud germination, stem elongation, and leaf development. It affects root growth and differentiation.

(Table 1) cont....

Plant Growth Regulator	The Part of the Plant where the Growth Regulator is Synthesized	Effect of Growth Regulator on the Plant
Abscisic Acid	Synthesized in leaves due to water stress.	Inhibits growth, closes stomata during water stress, and prevents dormancy from breaking.
Ethylene	Synthesized in many tissues of the plant depending on the stress.	Increases fruit ripening, and suppresses some auxin effects.
Brassinosteroids	Synthesized in seeds, fruits, stems, leaves.	Inhibits root growth, inhibits leaf abscission, and increases xylem differentiation.

In order for a compound to be qualified as PGR, it must be formed in the plant, be transported from the place where it is formed to another place, manage or regulate different life events in the place where it is transported, and show these effects even at very low concentrations [18 - 20].

Among natural PGRs, ethylene is the most widely used growth regulator in the world with 23%, while auxin is in the second place with 20% and Gibberellins is in the third place with 17%. Of these, auxins, cytokinins and gibberellins are growth promoters; While dormins can be grouped as inhibitors, ethylene mostly plays a regulatory role in fruit ripening [21 - 23].

PLANT GROWTH REGULATORS

The terms “Plant Growth Regulators” or “Growth Regulatory Substances” include natural plant hormones as well as chemical substances that have been found to be effective on plant growth and development, the number of which has been increasing in recent years and can be obtained synthetically. While every “Plant Hormone” is a “Growth Regulatory Substance”, not every “Growth Regulatory Substance” is a plant hormone [6, 24].

Auxins

Auxins are one of the oldest used plant growth regulators in agriculture. These are substances that provide cell expansion and growth, and cell elongation, tissue development and root formation are promoted by them. Auxins, which are plant growth regulators, are synthesized by all higher plants and the most abundant auxin form is Indole-3-acetic acid (IAA) [25, 26].

Researchers have stated that IAA is the only naturally occurring auxin. Natural auxins occur mostly in the apical buds and leaves and descend from the apex in the plant. IAA is abundant in the growing tips of the plant (coleoptile tip, bud, leaf and root tip). After clarification of the chemical structure of auxin (Fig. 1), it was determined that many chemical substances that are more or less similar in

CHAPTER 4

Current Assessment and Future Perspectives of Secondary Metabolites

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Abstract: Herbal secondary metabolites have become more and more significant in recent years because of the negative side effects of synthetic medications used to treat a variety of illnesses and the growing demand for natural industrial products. Green chemicals are becoming more and more popular, particularly as a result of the negative environmental impacts of synthetic chemicals. Among these green chemicals, especially herbal products are used in the pharmaceutical industry, in the production of natural dyes, and in the production of herbal fragrances and flavor substances. The importance of these secondary metabolites has led in recent years to investigate the possibilities of increasing their production using tissue culture technology. Thanks to plant tissue culture, it is possible to obtain secondary metabolites more cheaply and efficiently, without being affected by seasonal fluctuations. Secondary metabolites are obtained from medicinal plants and are also named as phytochemicals, natural products, or plant constituents. The studies about the plant secondary metabolites have been increasing in the last 50 years. These molecules have a major role in the adaptation of plants to their environment and also in the defense system of predators; and response to environmental stresses.

Keywords: Green compounds, Medicinal use, Plant, Secondary metabolite.

INTRODUCTION

Although humans have used various plant secondary metabolites in medicine and pharmacology for thousands of years, the chemical analysis began nearly 200 years ago with the first isolation of morphine from *Papaver somniferum* [1].

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There have been significant developments in plant analysis in the last 20-30 years. Natural products isolated from plants are used for the discovery and development of modern medicines [2]. Chromatography, electrophoresis, isotope techniques, and enzymology have been effective in elucidating the exact regime formulas and the most important biosynthetic pathways [3, 4].

The primary metabolites (nucleotides, amino acids, and organic acids) are directly effective in the vital functions of plants. Although the secondary metabolites are not directly effective in the vital functions of the plant. In the early years when secondary compounds were studied, it was argued that these substances were 'functionless and waste substances' [5, 6]. In the following years, it was explained by many scientists that these components are very important for the defense system of plants [7 - 13].

The metabolite name is usually limited to these small molecules, which are products of metabolism. The metabolites have various functions in plants such as fuel, structural, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity of their own (a cofactor), defense system, and interactions with other organisms. Plants produce a wide variety of organic compounds, the vast majority of which are not directly involved in growth and development [14, 15].

Secondary metabolism refers to metabolic pathways and distribution of minor process products that, unlike primary metabolism, are not necessary for the growth and reproduction of the organism [16]. The secondary metabolites are produced in the secondary metabolic pathways of plants (Fig. 1). Plant secondary metabolites have a huge group of various structure compounds [17].

Classification of Plant Secondary Metabolites

Plant secondary metabolites are generally classified according to their biosynthetic pathways [19]. A basic classification of secondary metabolites includes three main groups: terpenes (such as plant volatiles, cardiac glycosides, carotenoids, and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins, and lignin) and nitrogen-containing compounds (such as alkaloids and glucosinolates) (Fig. 2) [20].

Terpenes are one of the largest classes of natural products and more than 22,000 compounds belonging to this group have been identified [21]. While there may be terpenes that contain only hydrocarbons, there are also terpenes that contain oxygen. Oxygen-containing terpenes are also called "terpenoids" [22].

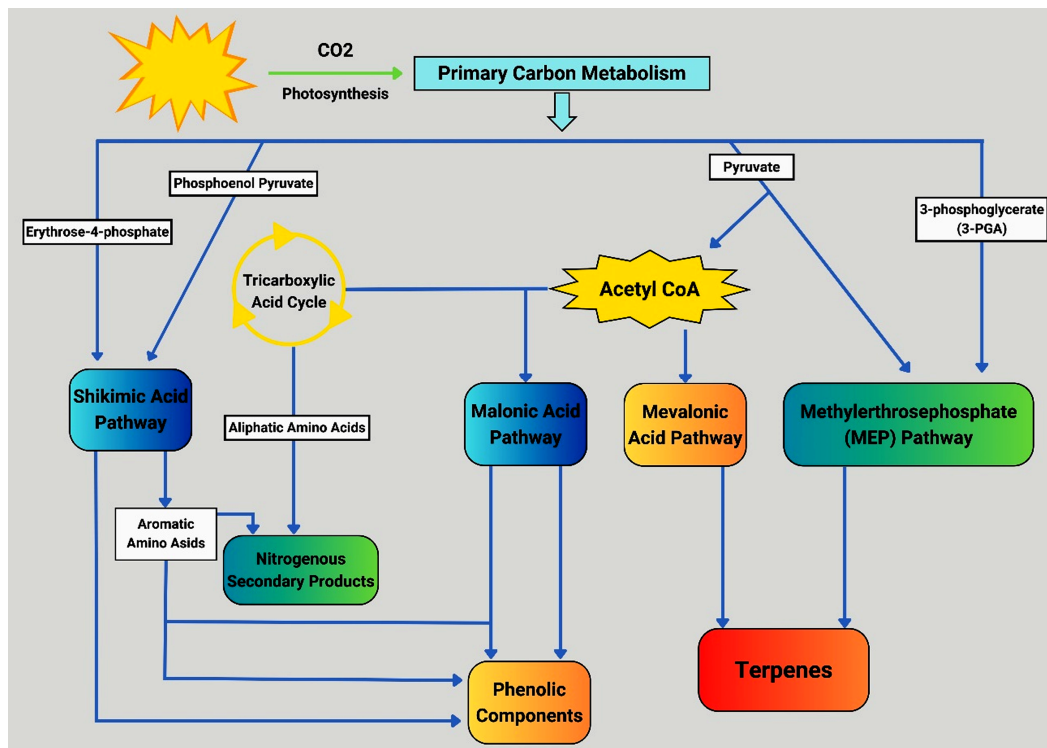


Fig. (1). Simplified view of the main pathways and primary metabolism of the secondary metabolites biosynthesis [18].

Terpenoids have various functional roles in plants. Examples of these are phytol and carotenoids as photosynthetic pigments, ubiquinone and plastoquinone as electron carriers, abscisic acid as hormones, and sterols as structural components of cell membranes [24].

Most terpenoids are lipophilic and readily interact with biomembranes and membrane proteins. They increase the fluidity and permeability of membranes, leading to uncontrolled flow of ions and metabolites and even cell leakage, which can ultimately cause necrotic or apoptotic cell death [25]. In general, terpenes are cytotoxic to bacteria and fungi, insects, and vertebrates and are used against infections [26]. Compounds in this group are very important in terms of odor potential [27]. The recent biological activities of some terpenes are given in Table 1.

Bioreactor Systems: Physiology of Cell Cultures

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Abstract: Cell culture in plants is a technique in which cells of plant tissues are developed *in vitro* in an artificial environment suitable for growth and proliferation. By developing different cell culture environments, it is possible to conduct many experimental studies such as cell proliferation, differentiation, identification of growth factors, understanding the mechanisms underlying the normal functions of various cell types, cell-cell or cell-matrix interactions, and determining the effects of molecules thought to be effective in metabolic pathways. Therefore, cell cultures have become one of the major tools used in cellular and molecular biology. Bioreactor systems, developed as an alternative support to traditional cell culture studies, aim not only for large-scale mass propagation, but also for the application of various physiological approaches, especially in plant protoplast cells, understanding metabolic pathways, and the factors effective in secondary metabolite production, and especially the application of transformation-oriented methods. In this context, this chapter aims to examine the physiology of cell cultures on a bioreactor basis and shed light on physiological processes with current and/or future approaches.

Keywords: Bioreactor, Metabolic pathways, Plant growth regulators, Protoplast.

INTRODUCTION

Bioreactors can be defined as mechanical containers or tanks in which living organisms, cells or tissues are cultured in a liquid nutrient medium and the conditions inside are kept under strict control. The term bioreactor is often used synonymously with fermentor. However, fermenter has a narrow meaning used to obtain alcohol from sugar in an anaerobic environment. The main difference that distinguishes bioreactors from traditional chemical reactors is the control and support of living-biological contents [1, 2]. Bioreactors can also be defined as a tool or device used to carry out one or more biochemical reactions to obtain the desired products from a starting material. Bioreactors represent the latest steps in

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the development of biologically based processes. In general, the basic function of the bioreactor is to provide optimal conditions for effective cell growth and metabolism by tightly regulating various key environmental (chemical and physical) factors [3, 4].

A bioreactor is a tank with an electronic control panel where probes that detect pH, temperature, and dissolved oxygen in the culture medium are placed, allowing the addition of fresh medium and removal of products (according to the operation mode), pH regulation, air supply, mixing and temperature control without disturbing the aseptic conditions of the culture medium. Thus, the bioreactor is a technological system with mechanical and electronic features that enable close monitoring of culture conditions and carry out the necessary physical and chemical interventions as programmed [5, 6].

The first bioreactors used outside microbial technology in plant cell and tissue cultures were stirred tank reactors. Various types of bioreactors have emerged with their application to plant cell and tissue cultures. Stirred tank reactors provided mixing and aeration by mechanical means. It was also used in bioreactors in gas-blowing units (Fig. 1) [7]. As we have emphasized several times before, plant secondary metabolites are produced in plant cell and tissue cultures in lower amounts than they are produced in the plant. For this reason, bioreactors of different shapes are trying to solve these problems and produce them at high yields. However, this technology has various problems that need to be solved in the production of secondary metabolites of commercial and medical importance. There are various factors that affect the production of bioreactors such as carbohydrates in the nutrient medium, nutrients, growth regulators, pH, atmospheric gases, oxygen supply, CO₂ cycle, viscosity - liquid medium fluidity, cell density, lysis stress of plant cells [8, 9].

Today, bioreactors are seen to be effectively applied in culture types such as cellular biomass production, organ cultures, somatic embryo production, and micropropagation. Liquid culture media increase the uptake of nutrients in micropropagation, and yield by encouraging growth, and provide a larger volumetric area. *In vitro* culture in liquid media also has disadvantages. Effects such as oxygen deficiency (asphyxia), hyperhydricity and shear forces can be considered as disadvantages. Considering these problems, modifications, combinations and the development of new bioreactor systems have been made in bioreactors. In micropropagation and organ cultures, systems (immersion bioreactors SETIS™, RITA®, WE VITRO) that contain two separate tanks and allow plant materials to come into contact with nutrient solutions for certain periods of time have been used in micropropagation and organ cultures in recent years (Fig. 2) [7]. Although bioreactors serve different application areas, they are

important biotechnology applications that will develop in many areas in the next century [11, 12].

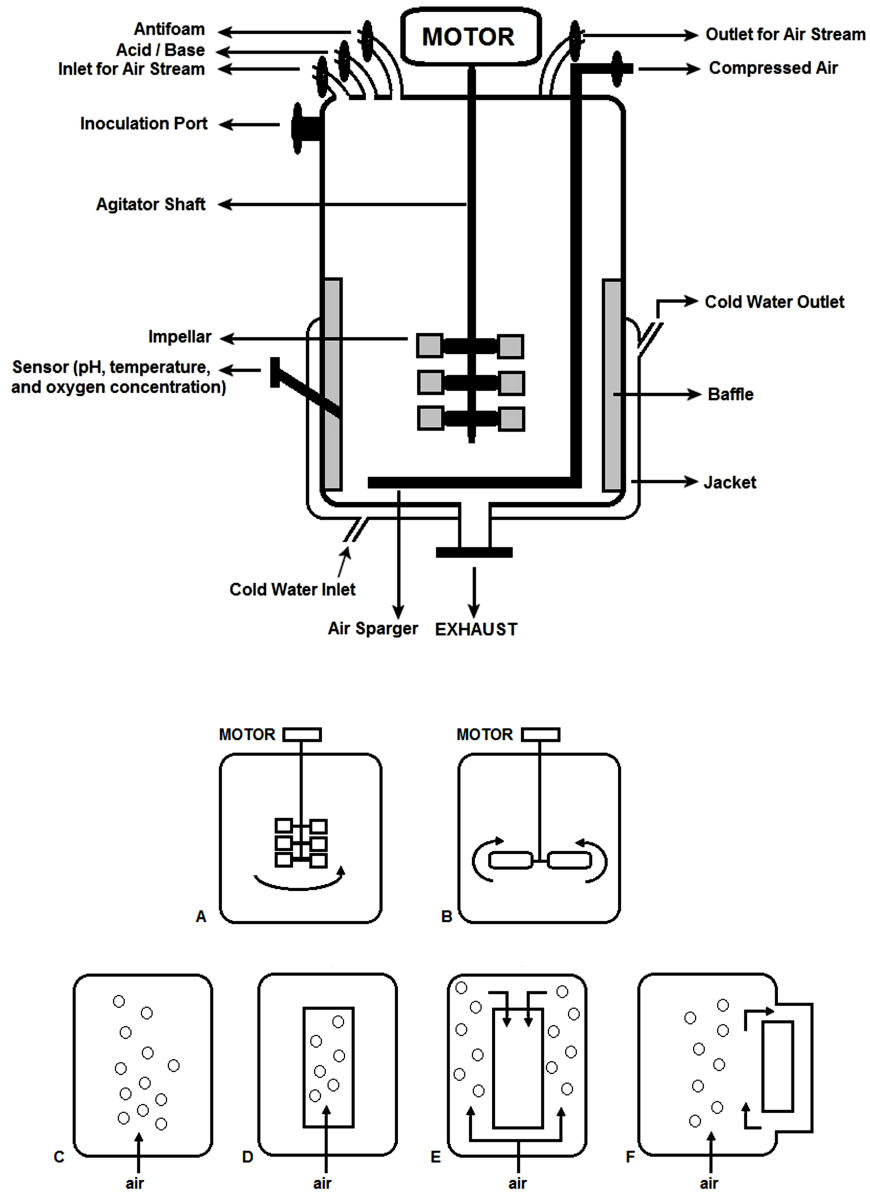


Fig. (1). Typical structure of a stirred-tank bioreactor system (upper side). Structure of different types of bioreactor systems. Flat-blade turbine impeller (A) and marine propeller (B) agitation based bioreactor systems, bubble column (C, D), draft tube air lift (E) and external loop air-lift (F) bioreactor systems (lower side) [7, 10].

Genomics - Proteomics Approaches in Plant Physiology

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Abstract: Today, climate change, the impact of which is felt more and more due to global warming, also affects food security. Increasing population and global warming cause challenges in food demand and medicine supply. New approaches need to be developed to tackle these challenges and maintain the current balance. In particular, plant physiology studies have been carried out in many fields since the beginning of human life due to its vital importance. With modern technologies, plant physiology studies have moved to a higher level. Studies at the molecular level, known as genomics and proteomics, are progressing towards the cultivation of plants with superior properties such as being more resistant and having higher nutrient content. The use of genomic techniques such as whole genome sequencing, comparative genomics, molecular markers, and proteomic approaches such as gel-based, affinity, and reagent-based, mass spectrometry has led to the acquisition of comprehensive data in the field of plant physiology. These data have led to a better understanding of plant morphology and development, the analysis of the complex interactions between genes and proteins, and the collective development of genetic-based breeding efforts. In this chapter, basic genomic and proteomic approaches in the field of plant physiology and past and present studies on economically important plants are presented.

Keywords: Comparative genomics, Metabolomics, Stress physiology, Transcriptomics.

INTRODUCTION

Genomics, which can be summarized as all the studies carried out to explain the organizational-functional information about the genome and genes, mediates the identification of all genes in a living thing separately, the investigation of the interactions of genes with each other and the environment, and the examination of the production and activation of genes in time, place and quantity. It is a branch of

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science that deals with advances in the fields of genomic automation and bioinformatics, which processes, interprets and stores its outputs through information technologies [1, 2]. Genomic studies allow the identification of genes related to disease and physiological processes, revealing structural-functional interactions between genes, and the roles of genes in development and their expression profiles, and comparing different organisms on a genetic basis [3, 4].

Considering that cell functions are regulated by proteins rather than genes and mRNA and the non-linear relationship between mRNA and proteins, it would be useful to supplement transcriptomics with information about proteins. Indeed, genomic data need to be supported by post-genomic studies to understand the extent to which the organism uses genes, which ones it responds to the situations it encounters, which genes are expressed and converted into proteins, their locations in the cell and their relative amounts (Fig. 1). This discipline, which aims to provide collective information about proteins, is called proteomics. Genomic studies are carried out through traditional and high-throughput methods [5, 6].

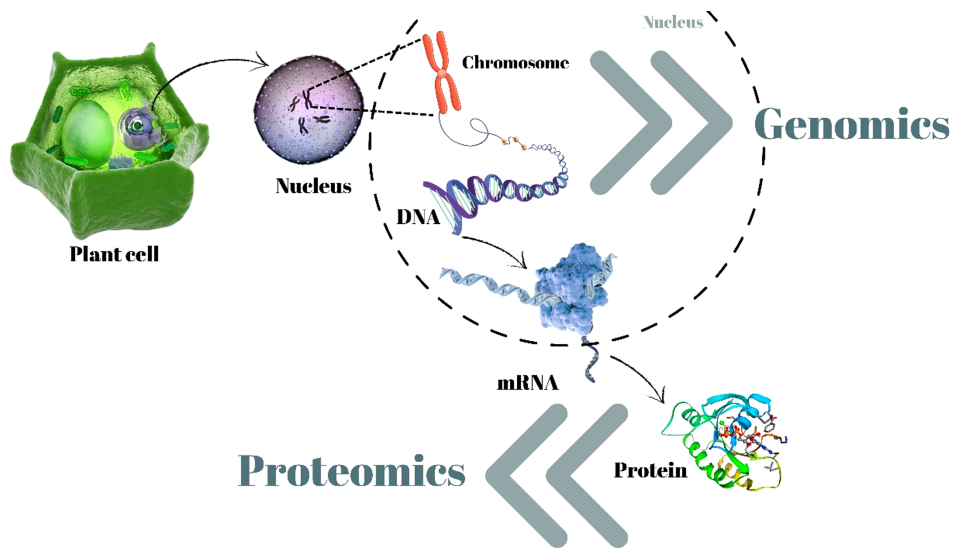


Fig. (1). Genomics and proteomics: Two complementary perspectives on life.

The proteome describes all proteins encoded by the genome. It is the entire set of proteins possessed and expressed by a given organism and includes not only the polypeptide structures encoded by genes but also post-translational modifications. The term proteomics refers to processes such as the expression of different proteins in different cell types and different cell parts and different developmental

stages, environmental conditions, various diseases, and aging. In other words, the proteome is a dynamic structure that varies according to tissues and cells, phases of the cell cycle, internal and external stimuli, environmental conditions, and similar situations. Proteomics aims to define the proteome. It refers to the examination of the proteome in the context of structure, location, quantity, post-translational modifications, function in tissues/cells, and interaction with other proteins and macromolecules. Unlike genomics, proteomics, which is a dynamic concept, can also be defined as the quantitative analysis technology of proteins in cells, tissues, or body fluids under different conditions [7 - 9].

Proteomic studies, which enable comparative studies, as in genomic studies, provide supporting information for genomics in situations that limit the effectiveness of genomics. The difference of proteomic studies from traditional biochemical methods is that they enable the study of a large number of proteins (proteome) simultaneously. Various methods can be used for proteomic studies that need strong bioinformatics support to interpret and make sense of the data it provides. However, studying proteins is not as easy as studying genes due to various reasons such as they derive certain characters from their three-dimensional structures. They are sometimes found in very low amounts, there is no clear relationship between their behavior and quantities, there are reversible post-translational modifications, and they can form splice variants due to RNA splicing [10, 11].

GENOMIC APPROACHES IN PLANT PHYSIOLOGY

Genomics is an interdisciplinary field that studies genome structure, function, mapping, evolution, and regulation. Genomics also includes the sequencing and analysis of genomes through the use of high-throughput DNA sequencing and bioinformatics to assemble and analyse the function and structure of whole genomes.

With modern technologies, the study of plant physiology in the field of genomics has progressed to a much higher level. Especially recently, the genome sequences of many plant species have become the main theme in plant physiology research. Open access and continuous updates create a fertile environment for the development of economically important plants. Recent technological advances and especially agricultural challenges have led to the emergence of high-throughput tools for research and utilisation of plant genomes for crop improvement. These genomics-based approaches aim to decrypt the whole genome, including gene and intergenic regions, to understand plant molecular responses and to develop strategies that enable improvement in many areas under plant physiology.

CHAPTER 7**Seed Physiology: Future Applications and Current Limitations****Ergun Kaya^{1*}**¹ *Muğla Sıtkı Koçman University, Faculty of Science, Molecular Biology and Genetics Department, 48000, Mentеше, Muğla, Türkiye*

Abstract: The basis of the evolutionary development of a plant is reproduction. Because reproduction ensures the continuity of species, therefore, immediately after fertilization, the seed becomes the recipient of substances assimilated in the plant. As growth continues, the seed basically goes through three main physiological stages. The period in which 80% of the growth occurs is the period of cell division and elongation, the supply of nutrients from the mother plant through the funiculus, and the intense increase in seed weight. The funiculus degenerates and the seed breaks its connection with the parent plant. The seed has reached the maximum dry matter. This period is called mass maturity. Although physiological maturity has been considered for many years, it has been determined that the seed is not physiologically mature during this period. In the drying period, the seed begins to dry. During this period, environmental conditions, rain, temperature, diseases and pests to which the seed is exposed reduce the quality. It is imperative to carefully combine advances in seed management, vegetative propagation, biotechnology, and molecular genetics to support forestry practices that strive to balance socioeconomic requirements, biodiversity, and climate change with sustainable production. The production, preservation, and repair of seeds all start with seed physiology and technology. This chapter aims to explain the physiological processes that affect the formation, growth, and development of seeds, which are the basis of the continuity of plant generations, by evaluating the perspectives of future applications and current limitations.

Keywords: Absciscic acid auxin, Cytokinin, Ethylene, Gibberellic acid.

INTRODUCTION

A seed is morphologically defined as a fertilized egg. This occurs as a result of double fertilization. Firstly, the endosperm is formed by the fertilization of one of the polar nuclei, and the embryo is formed by the fertilization of the egg cell [1, 2]. After double fertilization, endosperm development begins before embryo development. First of all, with the supply of energy, the absorption of nutrients

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from the main plant cells begins. The outer surface of the endosperm is covered with the aleurone layer, which is a layer containing high levels of protein. This layer plays an important role in enzyme synthesis during the germination period. The aleurone layer is a well-developed layer in grasses and lettuce but is less present in other species [3, 4]. In some species, the endosperm completes its development early and the nucellus ($2n$) is filled with nutrient tissue. With this, it meets the energy needs of the seed during the germination period. Beetroot and spinach can be given as examples of these species. During seed maturation, this tissue is called perisperm [5, 6].

Some seeds do not contain endosperm or have no perisperm. These seeds are called nonendospermic seeds. These types of seeds contain large embryos. These families include Fabaceae, Cucurbitaceae, and Asteraceae. The endosperm typically contains large amounts of starch, an energy-supplying compound. They contain lower amounts of storage proteins or some types of fat [7, 8].

With the fertilization of the egg, a zygote is formed, which ultimately gives rise to the embryo. Cell division does not occur without the formation of some endosperm. In the first stage, two cells are formed, the one closest to the micropile is elongated and more dominant. The cell in the upper layer ultimately gives rise to the embryo. The circuit of several cells is called proembryo. During this period, the embryo goes through four separate stages. The cotyledons of dicotyledon seeds are quite large. While endospermic seeds have thin, delicate and leaf-like cotyledons, non-endospermic seeds (peas, cowpeas and beans) have very large cotyledons and constitute 90% of the dry weight of the seed [9, 10].

As a general rule, the cotyledons that remain in the soil (hypogeal) when germinated are larger, and the cotyledons that rise to the soil surface (epigeal) are smaller. It can shed the seeds of some species before they are fully mature in terms of development. For example, carrot seeds leave the plant with an immature embryo and continue their development later [11, 12].

Under normal conditions, the seed moisture content is 80% at fertilization. Depending on the characteristics of the seed during the harvest period, it may drop to 50-60% in recalcitrant species, 20-25% in sub orthodox species (intermediate species, when kept at -20°C , the intermediate seeds from this plant may only last five years. They also have a tendency to deteriorate more quickly than traditional seeds. When they are dried between 45 and 65% RH, they last the longest), and up to 10% in orthodox species (This plant's seeds can withstand freezing temperatures and drying to an interior seed moisture content of less than 10%). The source of assimilates necessary for seed development is the mother plant. Starch formation occurs from the use of sucrose produced during

photosynthesis. In oilseeds, sucrose changes through some chemical reactions. Protein synthesis occurs by transporting amino acids such as asparagine and glutamine from the roots and leaves [13, 14].

Sugar, fat, and protein accumulation occurs equally throughout the seed development period. During the development period, the seed goes through two important periods in terms of dry matter accumulation: Physiological mass maturity: This is the period when the seed has the maximum dry matter. This period has been determined as the period when seed quality reaches its maximum level in some species [15, 16]. However, in some species, this period occurred one or two weeks later. Harvest maturity is the period when the seed is suitable for machine harvesting. It is also known as the period when seed moisture is 12-20%. It is especially important for dried fruit species. Looking at the changes in seed moisture during the development period, we can say that seed moisture decreases from 55-80% to 10-12% or 35-45%. It decreases to 10-12% in dried fruits and to 35-45% in juicy fruits [17, 18].

STRUCTURE AND DEVELOPMENT OF SEEDS

In general, the seed is the formation of the embryo through the division of the zygote in plants. Although the embryos look different in monocots and dicots, their developmental structures are the same, but the development of endosperm and other tissues is different. There are structural differences in the mature seed. The developing seed receives water and nutrients to accelerate cell division and elongation. Initially, the amount of sucrose and other sugars in the shell and endosperm is high, which are then converted to starch. Protein accumulation in the endosperm increases proportionally with time [19, 20].

Plants produce starch, also known as amyllum, which is a polysaccharide that is produced and used as a renewable source of industrial raw materials. Starch is found in the center of the food and feed chains. Two different types of glucose polymers make up starch: amylopectin, which is highly branched and contains shorter α -1,4-linked glucan chains joined by α -1,6-glycosidic bonds, and amylose, which is linear and moderately branched and comprises glucose moieties linked together by α -1,4-glycosidic connections. Higher plants create two types of starch: transient starch made in chloroplasts in photosynthetic tissues and long-term storage starch made in amyloplasts in non-photosynthetic tissues including seeds, storage roots, and tubers. During the day, transient starch produced from photosynthates is broken down at night to support metabolism and supply energy. Long-term storage ensures that storage starch is ready for remobilization during germination, sprouting, or regeneration [21 - 23].

Genetic Transformation: Current Opinion And Future Prospect

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Abstract: Nowadays, it is possible to transfer desired foreign genes into the genomes of various plant species in a stable manner through genetic engineering applications. Through genetic transformation, plants can gain resistance to diseases, environmental pressures, and various chemical compounds such as herbicides and pesticides. Numerous genetic transformation procedures, primarily involving the transport of exogenous genes and the regeneration of transformed plants, have been continuously discovered and improved for high efficiency and convenient manipulation. The delivery of biomolecules by nanomaterials has piqued the curiosity of researchers in recent years. To improve crops, plant molecular biology breakthroughs must be translated into an effective genotype-independent plant transformation system. Improving the nutritional quality of plants is another important advantage of genetic transformation. Genetic transformation studies on plants started in the 1980s, and effective genetic transformation methods have been developed until today. These methods are generally divided into direct transformation and indirect transformation. Each of the developed techniques has some advantages and disadvantages. When deciding which method to use in transformation, it is very important to consider these advantages and disadvantages and to choose the most appropriate method for the plant to be gene transferred for a successful transformation. In this context, this chapter is aimed at explaining genetic transformation methods in plants in detail, the developments in genetic transformation from the past to the present, and the expectations about the genetic transformation process in the future.

Keywords: Agrobacterium, Genetic engineering, Genetic transformation, Genetic transformation, Transgenic plant.

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INTRODUCTION

Humans benefit from plants in many fields, such as food industry, animal feed, medicine, soft drinks, paints, cosmetics, perfume industry, and dye industry. Today, serious problems occur in plant resources, which are very important for living things, due to reasons such as the increasing world population and abiotic and biotic stress factors in agricultural areas. Classical breeding studies have been carried out for centuries to address these problems such as the slow pace of breeding cycles, and genetic linkage drag. With classical breeding methods, it is possible to select the desired traits from existing plants and combine these traits to obtain new plant varieties [1, 2].

However, the yield increase obtained from classical breeding methods is not sufficient today due to the decrease in arable land, the need for large plant populations, and therefore the increase in cost and labor requirements. In addition to the desired traits in classical plant breeding methods, undesirable traits also manifest themselves in the plant. This situation has revealed the necessity of investigating new technologies in plant breeding studies [3 - 6].

Genetic transformation methods are used to improve a plant with a certain trait without changing other traits [7, 8]. In molecular biology, the term transformation refers to the removal of a foreign gene from the cell membrane and incorporation into the genome of the host cell [9, 10]. The foreign gene transferred here is called a 'transgene', and organisms that have successfully transferred the gene are called 'transgenic' [11, 12]. With genetic transformation based on recombinant DNA technology, it is possible to increase the nutritional value of plants, provide resistance to biotic and abiotic stress factors, or study plant metabolism. Many parts of the plant, such as the stem, leaf, embryo, callus suspension, protoplast culture, cotyledon, or reproductive cells, can be used in transformation studies. Since the 1980s, this technology has been used in tomato [13], rice [14 - 19], wheat [20, 21], tobacco [22, 23], grape [24], maize [25 - 27], cassava [28], canola [29], and petunia [30]. The transferred genes reduce losses caused by stress factors and increase crop yields [31, 32, 12].

Transformation methods developed for transferring foreign DNA into target plant cells are generally divided into direct transformation and indirect transformation methods [33, 34]. In indirect methods, a bacterial cell is needed to transfer the foreign DNA into the plant cell, while in direct transformation methods, genetic transformation is performed without the need for an intermediary cell (Fig. 1) [34, 35].

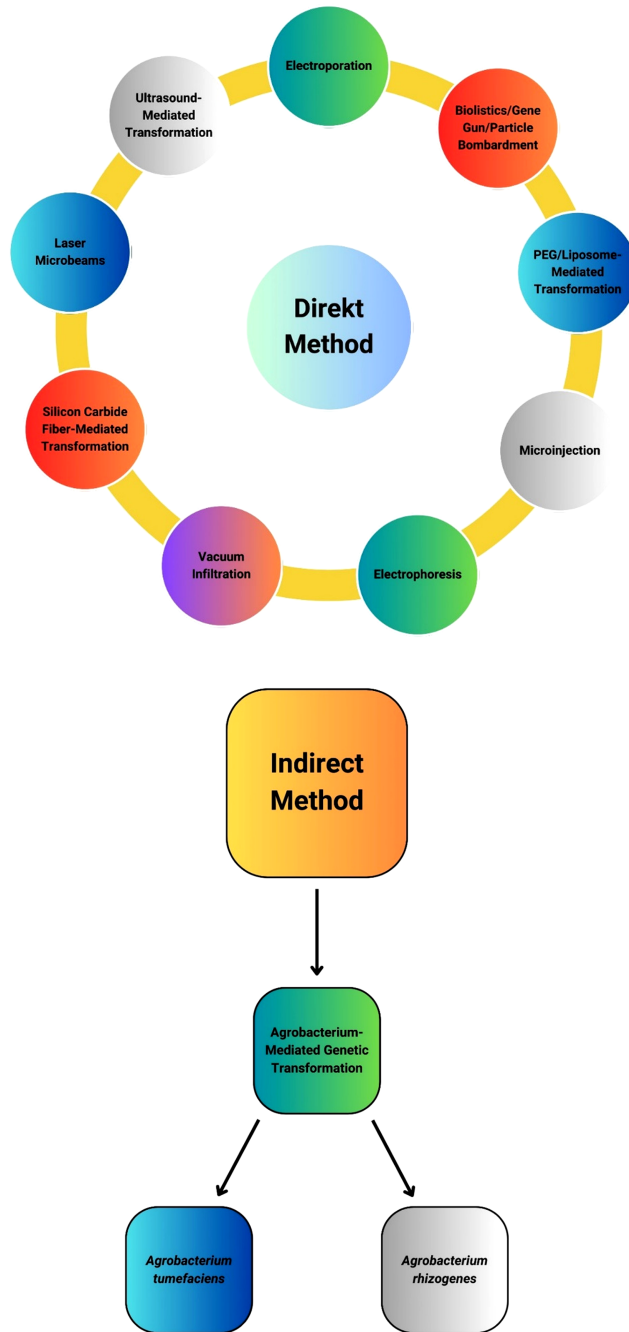


Fig. (1). Illustration of direct and indirect transformation methods.

CHAPTER 9

Crispr-Cas Technology: Targeted Genome Editing in Plant Physiology

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Abstract: The phenomenon of global climate change poses a significant threat to global food security, primarily due to the limited adaptability of major staple crops and plant species to the changing climatic conditions. This poses a significant challenge for farmers, agricultural experts, and policymakers worldwide as they seek to develop sustainable solutions to ensure adequate food supply in the face of climate change-induced threats. Significant improvement has been made to preserve crop yield, employing traditional breeding methods and cutting-edge molecular techniques to enhance the procedure. The utilization of CRISPR/Cas technology has recently gained traction as a viable alternative to transgenic methods in plant breeding. Our study in this chapter, for the first time, delves into the advantages of the CRISPR/Cas system in plant physiology, exploring key areas such as its impact on environmental factors, the underlying mechanisms of the CRISPR/Cas system, enhanced quality and yield, mitigation of biotic and abiotic stresses, ethical considerations, and regulatory issues, as well as the future prospects of this method.

Keywords: CRISPR/Cas system, Genome editing, Genetically modified organisms (GMO).

INTRODUCTION

Traditional plant breeding methods no longer suffice to address the challenge of feeding a growing population in a sustainable and productive manner. These methods are limited by reduced plant diversity, high costs, and time-consuming breeding processes [1]. Therefore, modern biotechnology and genetic engineering must be combined with traditional plant breeding. Genetic engineering is a complex area of biotechnology that involves the selection, location, isolation, purification, multiplication, and transfer of genes to create targeted changes and transformations in the genomes of plants and animals [2, 3]. While modern biotechnology holds tremendous promise for medicine, agriculture, and industry,

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safety concerns regarding genetically modified organisms (GMOs) and their products must be taken into account [4 - 6].

The practice of targeted genome editing in plant physiology holds vast potential for improving plant breeding. One such application is the modification of genes involved in growth and development, including those that affect the physiology of plants, which can increase crop yield [7 - 12]. Additionally, genes linked to disease resistance can be edited to bolster plants' natural defenses against pathogens, thus reducing the necessity for chemical pesticides [13 - 15]. Moreover, targeted genome editing presents an opportunity to enhance the nutritional quality of plants. By modifying the genes responsible for synthesizing crucial nutrients like vitamins, crop plants can be engineered to produce higher levels of these nutrients, which can help address nutritional deficiencies in human diets [16 - 20]. Generally, targeted genome editing has the potential to transform agriculture by enabling the creation of crops with improved traits, greater resistance to environmental pressures, and increased nutritional value [21 - 24]. However, it is crucial to approach this technology with responsibility, considering ethical and environmental concerns and implementing strict regulations to prevent unintended impacts on ecosystems and biodiversity.

New methods for improving gene targeting techniques, known as gene editing technologies, have recently been developed. These include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems [25 - 27]. Among these, based on advanced research, the CRISPR/Cas system is the most efficient, cost-effective, and dependable for gene editing purposes.

The CRISPR/Cas system is an advanced method of transgenic plant production that has been developed to address concerns related to crop quality and quantity (Fig. 1). This genome editing technique is capable of creating mutations in multiple gene locations and producing large deletions, thus enhancing the function and activity of plant genes and creating new traits [28, 29]. The CRISPR/Cas system has been widely used in animals and plants for gene silencing, gene replacement, multiple gene editing, identification of gene function, and regulation of the transcription process [30 - 32]. Studies on the CRISPR/Cas system have demonstrated its potential to accelerate plant breeding by boosting the enhancement of high-performance crops.

The utilization of CRISPR/Cas in plant physiology has numerous benefits, including creating disease-resistant crops and enhancing crop adaptability and resilience to varying environmental conditions like drought, extreme temperatures, and salinity. Moreover, it can improve crop nutritional value,

quality, and shelf life. Our study aims to provide a comprehensive view of how CRISPR/Cas9 is used in targeted genome editing of plants and its impact on plant physiology and transgenic plant development.

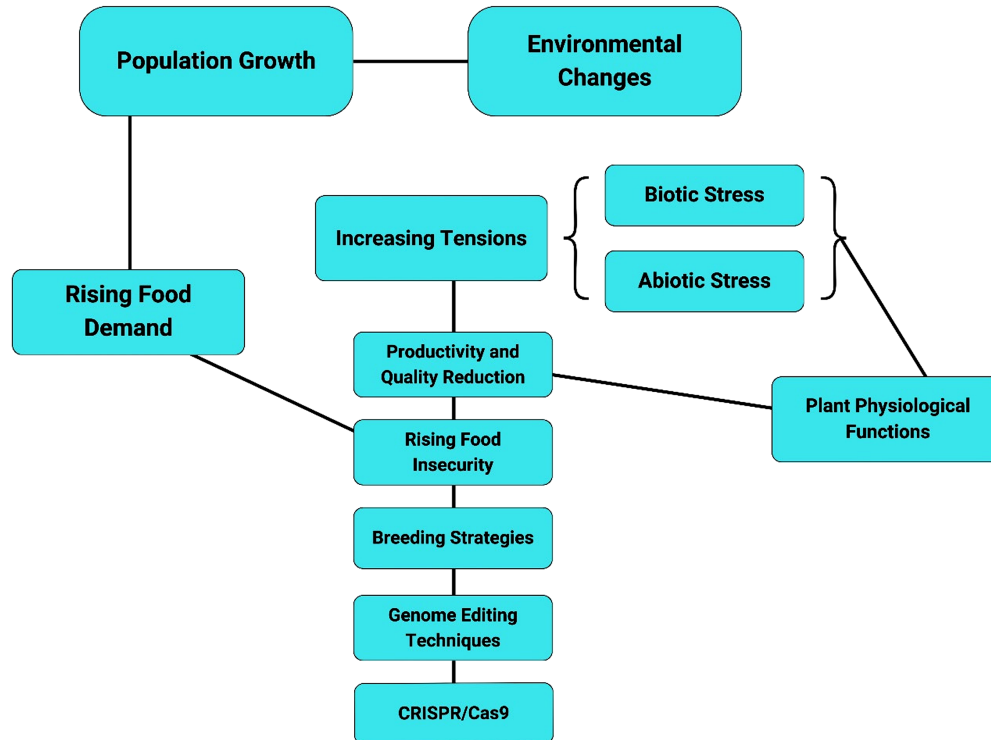


Fig. (1). Schematic overview of the CRISPR/CAS system's importance in targeted genome editing for plant physiology.

Precision Editing

Enhanced and accurate editing tools, such as CRISPR/Cas, which utilize numerous host DNA-repair paths to edit the genome in host cells, are needed to produce a wide range of plants with modified cells. However, achieving the highest efficiency and eliminating off-target effects poses a challenge. CRISPR/Cas system allows scientists to precisely target and modify specific DNA sequences within a plant's genome. It utilizes guide RNA to direct the Cas protein to the desired location, where it induces changes such as insertions, deletions, or substitutions [33, 34].

The system known as CRISPR/Cas was initially discovered in 1987 in the genome of *Escherichia coli*, functioning as an acquired immune system that

CHAPTER 10**Bioinformatics Approaches in Plant Physiology****Mehmet Emin Uras^{1,*}**¹ *Haliç University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Eyupsultan, Istanbul, Türkiye*

Abstract: Bioinformatics has proven to be a powerful tool in enhancing productivity across various fields, including plant biology. Bioinformatics provides significant capabilities for the acquisition, processing, analysis, and interpretation of large amounts of genomic data. With the help of next-generation sequencing technologies, large amounts of genetic data can be generated rapidly. The integration of bioinformatics tools into plant physiology allows the analysis of large amounts of genomic information, providing a better understanding of functional aspects of developmental, metabolic, and reproductive processes. Moreover, it offers a scientific framework for pre-experimental planning, in-experimental management, and post-experimental data analysis. The key applications of bioinformatics comprise gene and pathway identification, molecular docking, sequence analysis, RNA and protein sequence analysis and prediction, gene expression analysis, protein-protein interaction analysis, and statistical techniques that can be executed from genome to phenome. In order to enhance plants, bioinformatics may play a crucial role in encouraging the public release of all sequencing data through repositories, rationally annotating genes, proteins, and phenotypes, and elucidating links between the many components of the plant data. The integration of bioinformatics into plant physiology has the potential to facilitate crop improvement, identification, and/or development of new plant-based functional chemicals and biofortified functional foods and plants that are more resistant to stress conditions. Therefore, this contributes to a more comprehensive understanding in all areas of biology. These new approaches include pan-genomics, artificial intelligence, machine and deep learning applications, CRISPR technology and genome editing, single-cell RNA sequencing, third-generation sequencing systems, RNA engineering and post-transcriptional editing, and metagenomic studies. This chapter reviews the applications of bioinformatics methods in plant physiology and biological databases and their potential contributions to plant physiology.

Keywords: Artificial intelligence, Data analysis, Expression analysis, Genome, Metagenomics, Pan-genome, Protein-protein interaction, Single-cell RNA sequencing.

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INTRODUCTION

Computational science supports almost all areas of biology, and plant biology is no exception. Bioinformatics has become an important tool for acquiring, processing, analyzing, and interpreting vast amounts of genomic data. High-throughput technologies generate huge amounts of data, and the integration of bioinformatics tools and the vast data on plant physiology will provide a better understanding of the functional aspects of plants.

Bioinformatics provides a scientific platform for analyzing data before and after laboratory experiments [1]. The main applications in bioinformatics are gene and biological pathway identification, gene annotation, gene sequence analysis, RNA and protein sequence analysis and prediction, and expression analysis; statistics can be performed at the genomic level up to phenomics [2, 3]. Biological pathway identification [4], ncRNA detection [5], plant microbiome analysis [6], integrated phenomics [7], and proteogenomic approach to plant genomics [8] are good examples of recent studies in plant bioinformatics.

Plant physiology can be defined as the science of plant functions, including the dynamic processes of metabolic responses, regulatory processes, plant pathology, growth, and reproduction in living plants [9 - 11]. Important topics, like plant responses to biotic and abiotic stresses [12], production of primary and secondary metabolites [13], and biofortified plant breeding with genetic and epigenetic alteration [14], have gained importance in the area of plant physiology. Plant physiology mainly depends on biophysics, biochemistry, and molecular biology. Bioinformatics and plant physiology meet in molecular applications. The integration of bioinformatic tools into plant physiology offers opportunities for crop improvement, the discovery and development of new plant-derived functional chemicals, biofortified functional foods, and the discovery and development of industrial and energy compounds [15]. This chapter is a discussion of how bioinformatics can be a versatile tool for plant physiology (Fig. 1).

PLANT BIOINFORMATICS RESOURCES

The comprehension and regulation of plant physiological processes can be achieved through the use of biochemistry, biophysics, and molecular biology applications. However, integrating bioinformatic tools with these applications enables studies to become faster, more efficient, and more practical. One of the most important bioinformatics sources is databases, where genetic information is stored, and there are some tools for different analyses. This section provides an overview of key databases for molecular biology and bioinformatics research on plant physiology.

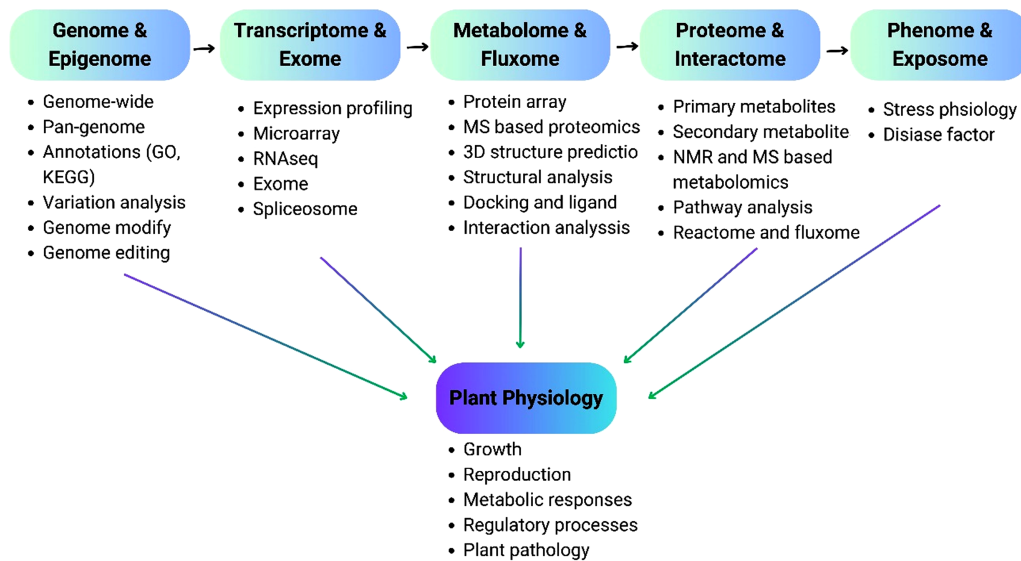


Fig. (1). Omics science and bioinformatics have the potential to advance research in plant physiology.

Major Genetic Information Sources

The National Center for Biotechnology Information (NCBI) GenBank database is a publicly accessible repository of nucleotide and protein information and is managed by the National Library of Medicine of the USA. GenBank collaborates with the European Nucleotide Archive (ENA) and DNA DataBank of Japan (DDBJ) in the International Nucleotide Sequence Database Collaboration. The databases exchange sequencing data to provide a comprehensive and standardized collection of data [16]. The database deposits nucleotide and protein sequences that have been submitted by authors and institutions globally. The sequence size can vary from a single coding or non-coding DNA region to an entire genome (NCBI, 2023). Coding sequences can also be translated to protein sequences. GenBank comprises several sub-databases, including Nucleotide, Genome, Sequence Read Archive (SRA), Gene Expression Omnibus (GEO), Structural Variation (dbVar, exclusive to humans), Single Nucleotide Polymorphism database (dbSNP, exclusive to humans), Reference Sequence Database (RefSeq), Conserved Domain Database (CDD) and Protein Clusters, among others. Sequences in GenBank are submitted as either single/multiple sequences by authors or as bulk submissions from genome survey sequences (GSS), whole genome shotgun (WGS), expressed sequence tags (EST), transcriptome shotgun assembly (TSA), and other high-throughput sequencing studies (HTS) (Table 1) [17].

Artificial Intelligence Technologies in Plant Physiology

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Abstract: Analyzing phenotypic traits, diagnosing diseases, and anticipating yields are just a few of the many applications of plant organ segmentation in precision agriculture and plant phenotyping. Because plant structures are so varied and intricate, traditional methods have a hard time keeping up. By combining several data sources, such as images and point clouds, graph neural networks (GNNs) have completely altered crop organ segmentation. In this research, we present a new method for rethinking plant organ segmentation by using the powerful features of GNNs. The approach takes a look at point clouds of plant shoots and uses graph representations to capture deep structural intricacies and intricate spatial interactions. One important novelty is the use of betweenness centrality for weighting edges and vertex, which guarantees that the segmentation results are biologically significant. The model's ability to understand geometric and topological details is improved, leading to more accurate segmentation through dynamic computing and continuous updates of Forman-Ricci curvatures. This all-encompassing work opens new doors for plant phenotyping research by improving the accuracy of organ segmentation and facilitating the integration of complicated mathematical theories into biological analysis.

Keywords: Artificial intelligence, Graph neural networks, Gibberellic acid.

INTRODUCTION

An essential part of precision agriculture and plant phenotyping, crop organ segmentation has recently been made much easier with the advent of Graph Neural Networks (GNNs). Using a variety of data sources, such as pictures and point clouds, crop organ segmentation identifies and categorizes distinct plant parts, including stems, leaves, and fruits [1 - 3]. Many applications rely on this method, such as phenotypic trait analysis, illness detection, and yield prediction. The complicated and diverse character of plant structures is often too much for traditional approaches, even though they are effective to some extent. GNNs offer

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a potential answer to these problems because of their capacity to grasp the complex spatial correlations in data.

A group of characteristics developed through the ever-changing interplay between genes and the environment is the focus of plant phenotyping research [4, 5]. The focus of traditional phenotypic studies has been on plots and individual plant levels measured by hand. However, newer multidisciplinary research on genomics and phenomics has highlighted the need for more precise and high-throughput phenotypic acquisition, particularly for organ-level traits related to ideal plant architecture, such as leaf and stem traits [6 - 8]. New insights into high-throughput phenotyping have been provided by developments in image sensing and processing technologies [9 - 12], which in turn enhance the accuracy of phenotyping at the organ level. As a result, the development of methods for efficiently and accurately extracting organ-level traits is an important yet emerging field.

The octree algorithm, Difference of Normals, and 3D skeleton were traditionally used in traditional approaches to plant organ segmentation from 3D data, like LiDAR point clouds [13 - 16]. Although these methods are able to segment a variety of crop species with varying leaf shapes and canopy structures, they are still not generalizable beyond a small set of plants with simple structures that can be fine-tuned by time-consuming and labor-intensive parameter adjustment. The cutting edge of plant phenotyping research right now is the development of a universal 3D segmentation method that can be applied to a wide range of varieties throughout different phases of growth.

The recent advancements in 3D deep learning techniques, along with breakthroughs in neural network topologies and large amounts of data, have the potential to greatly enhance the accuracy and generalizability of organ segmentation [17, 18]. In an effort to gain a better grasp of 3D data, several studies have concentrated on Multi-view [19 - 22]. According to Jin *et al.* [23], in order to accomplish direct segmentation on the point cloud, it is necessary to first partition the point cloud into a large number of voxels. Then, a 3D convolution can be employed. However, this approach demands a lot of computing power. Among the first end-to-end deep learning networks to work directly on points, PointNet [24] and PointNet++ [25] can perform object classification and semantic segmentation on points at the same time. The SPGN [26] uses the similarity of each pair of points in the feature space, for instance, semantic segmentation, and many other researchers have used similar frameworks to optimize and improve the feature extraction modules, leading to improved network performance. To better enhance the connection between local features of the point cloud, researchers have resorted to Recurrent Neural Networks (RNN) [27 - 29],

Conditional Random Fields (CRFs) [30 - 34], and Graph Neural Networks [35 - 40].

In this comprehensive study, we embark on an innovative journey to redefine the landscape of plant organ segmentation by harnessing the advanced capabilities of Graph Neural Networks (GNNs). Our approach marks a significant departure from the conventional paradigms and methodologies that have dominated the fields of deep learning and computer vision as applied to botany. At the heart of our method lies the strategic extraction of graph representations from point clouds generated from plant shoots, executed through four meticulously designed methodologies. Each of these methodologies is tailored to meticulously capture and interpret the intricate spatial relationships and structural complexities inherent in plant organs. This paradigm shift, from viewing plant organs as isolated entities to treating them as interconnected systems, enables our GNN framework to delve into the depths of plant structure and functionality. The result is a groundbreaking pathway to achieving more precise and comprehensive plant phenotyping, thereby revolutionizing our understanding and analysis of plant biology.

Our novel strategy emphasizes the critical role of betweenness centrality in the weighting of edges and vertices within our graph-based model. This sophisticated approach does more than just enhance the segmentation process; it redefines the way we understand plant architecture by integrating the principles of graph theory into biological exploration. By leveraging betweenness centrality to assign weights, we ensure that our model doesn't merely recognize the physical structure of plant organs but also appreciates their biological importance. This methodological innovation allows us to achieve segmentation outcomes that are not only accurate from a computational perspective but also deeply meaningful within a biological framework, providing insights into the functional connectivity and significance of different parts of plant organs.

Expanding the horizon further, our research introduces the dynamic computation and continual updating of Forman Ricci curvatures for both edges and vertices as part of the convolutional steps within the GNN framework. This adaptive mechanism is designed to refine and enhance the model's grasp on the geometric and topological nuances of plant organs throughout the segmentation process. By updating the Forman Ricci curvatures with each convolution step, our model maintains an acute sensitivity to the minute variations in plant organ geometry, thus facilitating a segmentation accuracy and depth of insight that was previously beyond reach. This cutting-edge application of Forman Ricci curvature not only elevates the precision of plant organ segmentation to new heights but also paves the way for the integration of complex mathematical theories into the analysis of intricate biological structures. Through this pioneering approach, we are setting

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