STRUCTURAL DEFENSE OF PLANTS AND PATHOGENESIS

A review written by 3rd Year Undergraduate Molecular Biology and Genetics Student at Izmir Institute of Technology

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Oct. 2023

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ENDORSEMENTS

This review by Berkay Ekinci, an undergraduate student in the Department of Molecular Biology and Genetics at Izmir Institute of Technology, is an excellent resource for those interested in the physical defense mechanisms of plants. Berkay has spent many hours poring through the research literature and summarizing a large quantity of material to present a clear explanation of the subject at a level that is accessible to all readers. In addition, he has provided his own photographs and drawings to enhance the text. The work reflects his sincere interest in the topic, his desire to delve into scientific literature and his generosity in sharing his knowledge with others.

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Prof. Dr. Anne Frary
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Department of Molecular Biology and Genetics, Faculty of Science

Plants are exposed to a vast array of pathogenic diseases and pests and nevertheless manage to survive thanks, in part, to their physical defenses. Understanding these defense mechanisms is key to identifying targets for improving crop stress tolerance. In this work, Berkay Ekinci has summarized the physical defense mechanisms of plants. He has reviewed all of the relevant literature and produced a valuable resource for readers of all levels.

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Prof. Dr. Sami Doğanlar
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Department of Molecular Biology and Genetics, Faculty of Science

This review is a reflection of Berkay Ekinci's dedication as an undergraduate student at the Department of Molecular Biology and Genetics at the Izmir Institute of Technology and demonstrates his commitment to explaining the complexities of plant defense mechanisms through comprehensive research and compilation. Berkay Ekinci devoted significant effort, selflessness, and passion to carefully creating every facet of this exhaustive source during his undergraduate years. From detailed drawings to photographs, each component of this study reflects his dedication, demonstrating the culmination of intense hours spent in preparation. I am confident that individuals interested in understanding the complex world of plant defense dynamics will find value in his work.

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ISBN: 978-975-6590-27-0

ACKNOWLEDGEMENTS

I am proud to be admitted to the IZTECH MBG family in 2020, where I am still a 3rd year undergraduate student.

Since June 2022, I have been an attendant of the Doğanlar&Frary Plant Laboratory Team with the approval of Prof. Dr. Sami Doğanlar and Prof. Dr. Anne Frary, who are respected faculty members. Until now, both the literature research I have done for a project presentation about downy mildew disease in grapevine, and the routine lab meetings have improved my curiosity as well as shown me how little knowledge I have in this field. For this reason, I felt the need to do extensive research to write a detailed review about plant immunology both to improve myself and to inspire these who are interested in this field like me.

The review, which I started writing in February 2022, took a long and tiring time because I wanted to add my own stereomicroscope shots and use my own figures. However, this first attempt at a professional review has been a very instructive and improving work for me as well. My work with over 120 pages includes 21 different figures drawn by myself and covers the constitutive structural/chemical defense mechanisms of plants, induced-immunity pathways including effector and pattern-triggered immunity of plants, pathogens, and pathogenesis. The reason of publishing the first 40 pages of my review on structural plant defense mechanisms is that the rest of it still needs to be edited.

I would like to express my gratitude and respect to Prof. Dr. Yusuf Baran, the Rector of IZTECH, who invited me to his office to meet me after I wrote an article on CRISPR-Cas9 for the GENOMLINE journal when I was in the 1st year (Ekinci 2022). He also inspired me about academic writing by gifting me his book on cancer molecular biology. I would like to express my gratitude to Prof. Dr. Anne Frary, who helped me improve my review by reading it, to Prof. Dr. Sami Doğanlar, who opened the laboratory and the stereomicroscope, which I used for photography in my review, for my use and gave me the opportunity to experience it, and to my supervisor Postdoc researcher Dr. Asena Akköse Baytar, who sincerely supported me in all my questions and problems.

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INTRODUCTION

The major challenge in today's world is ensuring an adequate food supply for the growing global population. Achieving this goal requires the development of crops by using sustainable agricultural strategies in ecologically suitable areas (Jan et al. 2011; Doğanlar et al. 2023). However, plant diseases, which are caused by various pathogens such as bacteria, fungi, viruses, nematodes, and herbivores, pose a significant threat to crop quality and yield. Fortunately, plants have several preexisting and induced defense and immune mechanisms to protect themselves against biotic and abiotic stresses (Jones et al. 2006; Freeman et al. 2008).

This review aims to provide information on phytopathogens, the steps of pathogenesis, plants' pre-existing structural defense mechanisms against pathogenesis, and more. It is aimed at broadening the reader's knowledge and perspective by providing a wide range of examples, from simple to complex. I hope that this review will be a good start for enlightening and inspiring all curious scientists who, like me, are enthusiastic about this field.

CHAPTER 1. PATHOGENS AND PATHOGENESIS

This chapter is a brief review of phytopathogens, their types, the necessary factors for pathogenesis and the fundamental principles of pathogenesis in plants.

1.1. Types of Phtytopathogens

Phytopathogens are organisms that infect plants and reproduce by absorbing organic compounds from plant cells. Phytopathogens have several strategies to infect plant cells. Due to that reason, they are classified into three main groups: necrotrophs, hemibiotrophs, and biotrophs.

Necrotrophs are pathogens that occupy and kill plant cells to supply their nutritional needs, such as sugars, amino acids, organic acids, phenolics, fatty acids, and enzymes. For example, *Botrytis cinerea* is a necrotrophic fungus that causes gray mold disease in many species, including grapes.

In contrast to necrotrophs, hemibiotrophs need living plant tissue at the beginning of their life cycle but then kill host cells to survive. *Magnaporthe grisea* is a good example of a hemibiotrophic fungus that causes rice blast disease.

Biotrophic pathogens need living, infected plant cells throughout their life cycles to supply their nutrient requirements. These pathogens are obligate parasites. *Erysiphe necator* and *Plasmopara viticola* that cause powdery and downy mildew, respectively, are good examples (Dou et al. 2012; Wen et al. 2012; Guttman et al. 2014 Pusztahelyi et al. 2015).

The following section will discuss the components required for interaction with pathogens to establish favorable conditions for pathogenesis, as well as the delicate balance between host defenses and pathogen adaptation.

1.2. Necessary Components for Infecting Plants by Pathogens

In order to serve as host cells, plants must be susceptible to at least one of four different conditions that are associated with the disease process: an abundance of pathogenic recognition factors that facilitate the adhesion of pathogens to the surface of plant cells; environmental factors that are unfavorable to the plant; weakened host’s immune system that results in

absence of pathogen recognition receptors in plant cells; or an abundance of essential nutrients and growth factors.

In order for a compatible interaction between host and phytopathogen to occur, environmental factors should favor the pathogen and work against the plant's defense system. To allow the attachment of pathogenic recognition factors, the defense mechanism of host cells must be weakened. This mechanism is normally responsible for detecting pathogens and triggering the plant's immune response. In addition, the host cell should contain essential nutrients and sufficient growth factors to create a suitable environment for the proliferation of pathogens (Surico 2013; Anderson et al. 2010; Brown 1980; Piña et al. 2012; Fatima et al. 2015).

The following section will discuss how recognition factors play a critical role in the specificity and success of pathogen-host interactions.

1.3. The Role of Recognition Factors

The recognition factors (also called host-specific toxins) of a pathogen must be compatible with the receptors of a host cell for cell-to-cell adhesion to occur. In other words, for successful infection of the plant by a pathogen, that pathogen's recognition factors should be specialized to interact with the receptors of the host cell (Boyle 2003; Van et al. 2007).

The following section will provide a comprehensive overview of the steps involved in pathogenesis for different types of pathogens, including bacteria, fungi, viruses, nematodes, and aphids.

1.4. Steps of Pathogenesis in Brief

Pathogenesis is the process of disease development caused by pathogens. It occurs in stages: infection, colonization, and reproduction (Leach et al. 2014). In general, the endophyte must first attach to a plant's favorable susceptible part(s). This is followed by pathogen morphological changes (formation of infection structures such as haustorium, and appressorium), that are used to penetrate plant tissues or access them through openings such as stomata (Grennan 2006; Zeilinger et al. 2015; Vidaver et al. 2004).

The entrance of the pathogen is followed by colonization in the apoplastic (xylem or intercellular spaces) pathway of the plant if it contains sufficient nutrients and hormones.

Pathogens secrete effector molecules in the apoplast that accelerate pathogenesis by negatively affecting the physiological and biochemical activity of plants (Hou et al. 2011; Zimaro et al. 2011).

The process of pathogenesis varies according to type of pathogen as described in the following sections.

1.4.1. Bacterial Pathogenesis

Pathogenic bacteria are called 'endophytes' due to the fact that they do not cause disease, at least for a while, during their endosymbiotic relationship with plants (Aamir et al. 2020). Bacterial pathogenesis can be examined in three main steps:

a. Insertion

Endophytes insert into plants through natural openings such as stomata, lateral roots, nectarthodes, stigma, hydathodes, and trichomes, or they can enter through wounds. Attachment is done via lipopolysaccharides, pili, or cell surface (adhesive) proteins that exist in the outer membrane of bacteria (Boyle 2003; Grennan 2006; Vidaver et al. 2004; Kandel et al. 2017; Melotto et al. 2008).

b. Finding a Host Cell:

Bacteria move into plant tissues, invade the apoplast, and become closer to the host cell with different movement techniques such as aerotaxis (depending on oxygen concentration), chemotaxis (movement toward chemical gradients such as sugars, isoflavones, and amino acids), and electrotaxis (electrical fields from roots help bacteria in move toward the host cell) (Boyle 2003).

c. Colonization

Colonization varies depending on whether it occurs on the surface of the plant, in the apoplast, or within a gall. Endophytes are capable of colonizing the surface of the plant (phylloplane, rhizoplane, carpoplane, etc.) via siderophores, which are found in some bacterial and fungal species. Siderophores are molecules responsible for the accumulation and transfer of iron between organisms; they also help bacteria adhere to the plant surface and colonize under suitable conditions (Zeng et al. 2010; Page 2019).

Bacteria and a few biotrophic fungi can reproduce in the apoplast, causing parenchymal-vascular diseases. The proliferation of pathogens in vessels blocks the passage of water in the xylem, thereby causing the death of leaves or the entire plant (Boyle 2003).

Galls are contorted and rounded tumor-like infected outgrowths that have proliferated and accumulated bacteria, fungi, or nematodes. These phytopathogenic knot-like colonized clusters can be several centimeters in size and may appear at the soil line on the root and lower stem, as well as on buds and bark. The galls are greenish in the beginning but turn brown or black. An example is crown gall disease, which is caused by several gall-forming bacteria such as *Pantoea agglomerans pv. gypsophilae* (which affects *Gypsophila paniculata*), pathovars of *Pseudomonas savastanoi* that infect olive and oleander, and *Rhizobium radiobacter* (also called *Agrobacterium tumefaciens*) which causes gall disease in plants of the grape-rose family, nuts, and garden plants (Jones et al. 2006; Anderson et al. 2010).

1.4.2. Fungal Pathogenesis

Water molds (oomycetes) and fungal pathogens can form adhesive infection structures or directly enter the plant via the epidermis to find a host cell (Zeilinger et al. 2015).

Fungal spores first move towards plants, aided by polarity and hydrophobicity (step 1 in Figure 1). The second interaction between the pathogen and the host occurs through adhesive proteins (step 2). On the plant cuticle, spores germinate by producing germ tubes, which are composed of long, thin filaments made up of β-linked glucans and glycoproteins (a germ tube is illustrated at step 3). These germ tubes are also referred to as *hyphae*. An appressorium (plural: *appressoria*) is a fungal hyphal extension that elongates from the germ tube (step 3). A higher concentration of polyethylene glycol inside the appressorium causes water to move into the appressorium. Additionally, various melanin pigments (including L-3,4-dihydroxyphenylalanine, dihydroxynaphthalene, etc.) bind between the appressorial plasma membrane and the appressorial cell wall to block the passage of extracellular osmolytes (water stress-regulating organic compounds such as anions, sugars. malate and its derivatives) intracellularly through the appressorium. As a result, the outflow of water molecules is obstructed, and the turgor pressure of the appressorium is increased. This hydrostatic force facilitates the penetration of the plant surface. This invasive activity is further aided by enzymatic activity. Enzymes (such as cellulase and cutinase) found in the extracellular matrix of appressoria damage the cuticle and perforate it via penetration hyphae (step 5). The

penetration peg is the initial point of perforation on the plant's surface by the appressorium. Subsequently, the fungal hypha differentiates into invasive hypha and branches into the plant epidermis intercellularly (Step 6) (Zeilinger et al. 2015; Chang et al. 2014; Chethana et al. 2021). When the invasive hypha reaches the host cells, it breaches the epidermal cell wall of plant cells and forms infection structures intracellularly (Step 7). Some of the infection structures, such as the haustoria, hyphopodia, and infection cushion, are present in bacteria, fungi and parasitic plants and absorb plant metabolites from the host cell (Meng et al., 2009).

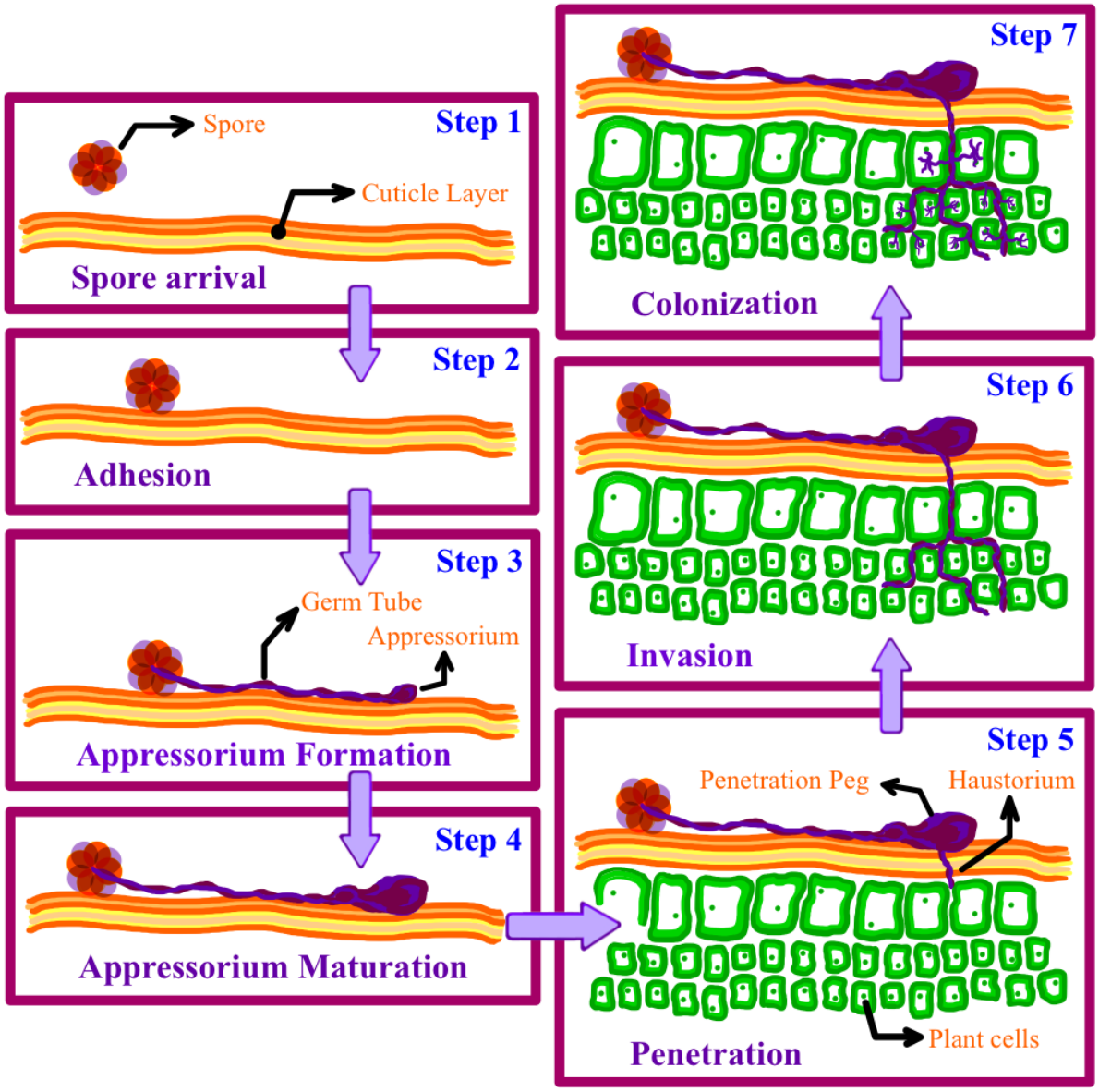

Figure 1. The steps of plant pathogenesis

1.4.3. Viral Pathogenesis, and Nematode and Aphid Pathogenesis

There are several ways for viral pathogens to enter plant cells. Viruses and virions can enter plant cells through wounds. Arthropods that feed on plant sap enable the passage of viruses into the plant vasculature. A bacterium that enters plant cells may carry the viral genome in its vector. Ultimately, both viruses and virions move through the symplastic pathway (symplasm) by passing intracellularly through plasmodesmata (Tabassum et al. 2012). During this process, host components such as virus-encoded factors and movement proteins regulate the movement of viruses within the host cell (García 2015; Martines et al. 2015).

Plant-parasitic nematodes (roundworms) and aphids (phloem-feeding insects) have a stylet that looks like a hypodermic needle. Similar to appressoria, stylet can penetrate the plant cells for invasion and nutrient uptake, or it can secrete sufficient metabolites to support parasitic and invasive activities (Lambert et al. 2002).

CHAPTER 2. Pre-existing Structural Defense Against Pathogenesis

Although plants do not have a complex immune system like mammals, they are able to recognize and protect themselves from pathogens through pre-existing and post-existing structural defense mechanisms (Sukno et al. 2008).

Pre-existing defense is the first level of defense before pathogen colonization and is also referred to as 'basal,' 'preformed,' 'passive,' or 'pre-invasive' defense. The plant's physical barriers, such as bark, cork, cell walls, wax layer, cutin, and cuticular lipid, passively arrest or slow down the passage of pathogens, allowing the plant to protect itself from colonization and invasion. These physical barriers also provide the plant with its physiological shape and strength (Soanes et al., 2007).

The following sections will explain the composition and structure of plant cell walls, including the primary cell wall, secondary cell wall, and middle lamella.

2.1. Plant Cell Wall Composition

The plant cell wall is composed of three main layers: the primary cell wall, the secondary cell wall, and the middle lamella.

2.1.1. Primary cell wall

The primary cell wall is composed of short and loosely arranged chitin, pectin, a relatively low amount of cellulose and a relatively high hemicellulose content, along with lignin (Figure 2.1). It lies between the middle lamella and the secondary cell wall. This layer forms in growing cells, hence it is referred to as "primary", and it is present in all plant cells. The primary cell wall is more elastic, thin and extensible than the secondary cell wall, and it contains a much higher water content.

While perforations are not found on the primary cell wall, pits are formed between the secondary cell wall of adjacent cells (pits and perforations are discussed in detail in section 2.7.1.). The primary cell walls do not always require modification, but once cell growth stops, the primary cell walls can be deposited within older layers to produce a secondary wall, thereby thickening the cell wall.

2.1.2. Secondary cell wall

The secondary cell wall has more than one layer (S1, S2, S3) with different compositions due to the deposition of the primary cell walls. Additionally, secondary cell walls have diverse microfibril orientations, including long and compactly arranged suberin, silica, resin, wax, a relatively high amount of cellulose, and low amounts of hemicellulose polymers (Figure 2.1). The cellulose microfibrils in the layers of the secondary cell wall are arranged in parallel. The most common polymer found within the secondary wall is *lignin*, which functions as a complex network of phenolic compounds. Lignin is found in xylem vessel walls and the fiber cells of woody tissues. These structures play a major role in providing skeletal support for plant structures and facilitating the movement of fluids. The secondary cell wall layers are located between the primary cell wall and the cell membrane. However, it is important to mention that the secondary cell wall is only present in mature cells; it does not exist in growing cells. It possesses a rigid structure and is not easily expandable, thus providing plants with stability and strength against mechanical stress (Brown 1980; Van et al. 2007; Alberts et al. 2002).

2.1.3. Middle lamella

The middle lamella (Figure 2.1) is composed of a cellulose synthase complex that holds the primary wall of adjacent cells together to form tissues. The complex contains a high calcium content, which is sufficient for cohesion. The middle lamella is the first layer of a cell wall to form after cytokinesis (Xu et al. 2010; Hansmann et al. 2003).

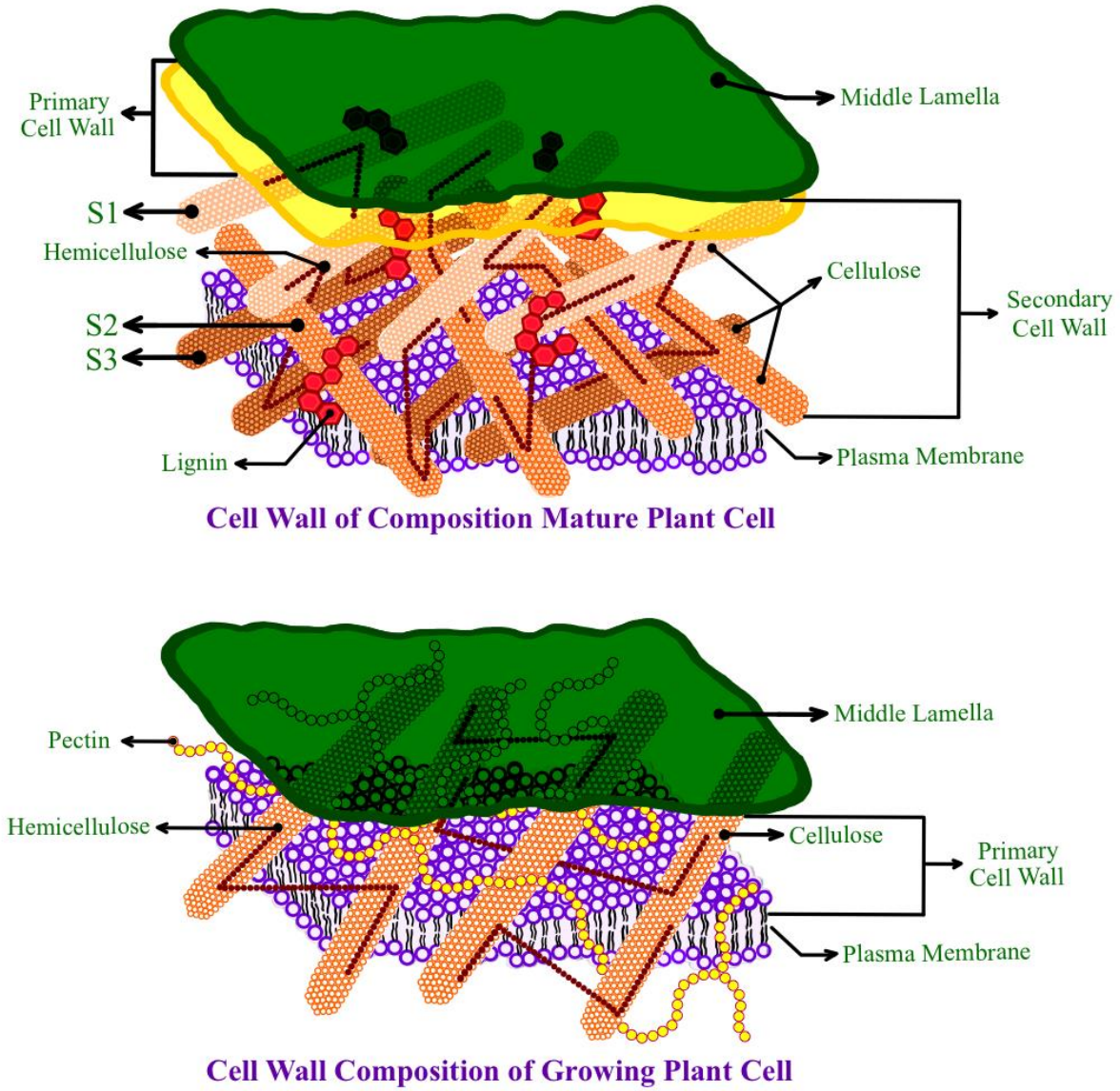

Figure 2.1. Cell wall structure and composition of mature and growing plant cells

2.1.4. Lignin

Lignin is an organic compound that is a complex and heterogeneous phenolic polymer. Lignin in the cell wall of mature plant cells (Figure 2.1). This polymer plays a crucial role in a plant's passive defense mechanisms. Three types of lignin polymers are found in different plant species: guaiacyl (G) lignin, syringyl (S) lignin, and p-hydroxyphenyl (H) lignin. The lignin fibers may exist in chemically modified form, appearing as a helical conformation or an annular ring. These lignin fibers are components of wood, plant vessels, tracheids, and the secondary cell wall. Lignin not only supports the trafficking of molecules in the xylem but also provides mechanical strength to the epidermal cell skeleton by thickening the plant cell wall, especially the secondary wall. The presence of lignin in the secondary cell wall is particularly important

because lignin molecules form an impermeable and rigid barrier against hyphal penetration (Brown 1980; Liu et al. 2018).

2.1.5. Hemicellulose and Cellulose

Organic glucose polymers, such as hemicellulose and cellulose, are found in both the primary cell walls and the secondary cell walls (Figure 2.1). These molecules interact with microfibrils to increase the flexibility and strength of the cell wall. They also help plants prevent the invasive activity of pathogens by covering the epidermis and blocking the route of water. As a result, pathogenesis is slowed down (Van et al., 2007; Xu et al., 1996).

2.2. Plasmodesmata

The plasmodesmata act as a microscopic bridge between adjacent cells, creating a pathway for cell-to-cell communication and intercellular trafficking of cytoplasmic materials. However, the plasmodesmata also serve as a passage for the spread of pathogens from one plant cell to another (Haywood et al. 2002).

The following section will cover tracheary elements, their characteristics, and the function of pits in xylem tissues. Additionally, the interaction between plant tissues in the xylem and pathogens, which may result in parenchymal-vascular diseases, will be discussed.

2.3. Xylem and Phloem

The plant vascular system has phloem and xylem tissues. The xylem tissues have differentiated water-conducting cells known as tracheary elements: tracheids and vessels.

2.3.1. Tracheary Elements: The Route of Pathogens

A tracheid (trachea) is an elongated xylem cell with interlocked tapered ends. They are the primary components of xylem tissues responsible for transporting minerals, salts and water. To briefly mention the difference of vessel elements from tracheids, a vessel element is wider, cylindrical cell that joins together with perforation plates instead of tapered ends (Figure 2.2).

Tracheid and vessel elements are dead cells due to their characteristic maturation stages (initiation, elongation during cell growth, differentiation, cellular aging and apoptosis). These functional cells form a network within xylem bundles. The tracheary elements also have lignified and thickened cell walls that provide mechanical support for the cell

skeleton. Because they have lignified cellulosic cell walls, the penetration of tracheary elements by pathogenic appressoria is more difficult than the penetration of a typical plant cell wall.

Although several pathogens have evolved to access phloem tissues, xylem tissues are usually a convenient route for pathogens. Once a pathogen successfully penetrates the thickened cell wall of the tracheary elements via its appressorium, the pathogen enters the xylem tissues. The pathogen inside the xylem tissues must pass through pits and perforations to reach the host cell. Another way to access the interior of the xylem tissue is to directly enter through the pits that surround the tracheary elements. To clarify what pits are, the formation of pits can briefly be explained in the following paragraph.

a. Pits Physiology and Formation of Pits

Pits are formed by the exosomal hydrolysis of the secondary cell wall of two adjacent tracheary elements. The non-degraded primary cell walls of adjacent cells form a pit membrane which is an elastic thin layer with two primary cell walls and a middle lamella (Figure 2.2).

Pits play a major role in water flow between tracheary elements. Pits can be opened and closed due to the heterogeneity of the pit membrane, maximizing the conductivity of xylem. Pits block the entrance of air into water-conducting elements to ensure the passage of water is not disrupted. The torus is a characteristic structure of pits that blocks the entrance of air (Figure 2.2). A thick and impermeable torus is located at the center of the pit membrane. The margo is another structure that surrounds the pit membrane borders with its cellulose microfibrils (Figure 2.2.). In other words, the edge of the torus is surrounded by the thin and permeable microfibrils of the margo. With altering air and water pressure, the margo moves towards one side of the pit due to its flexible structure, and the torus closes the pit opening (also called pit aperture, pit chamber), resembling a valve (Figure 2.2) (Chukhchin et al., 2021; Turner et al., 2007).

The pathogens that successfully access the interior of the xylem by penetrating the lignified cell wall of the tracheary elements or directly enter through the pits that surround the tracheary elements. These pathogens may cause parenchymal-vascular diseases, resulting in death of leaves or the entire plant (Boyle 2003).

Tyloses are parenchymal cell outgrowths that protrude through pits into xylem vessels when an infection is initiated. These growths physically block the xylem flow for slowing down the pathogenic processes (Jan et al., 2011; Jones et al., 2006; Freeman et al., 2008; Dou et al., 2012; Anderson et al., 2010; Van et al., 2007).

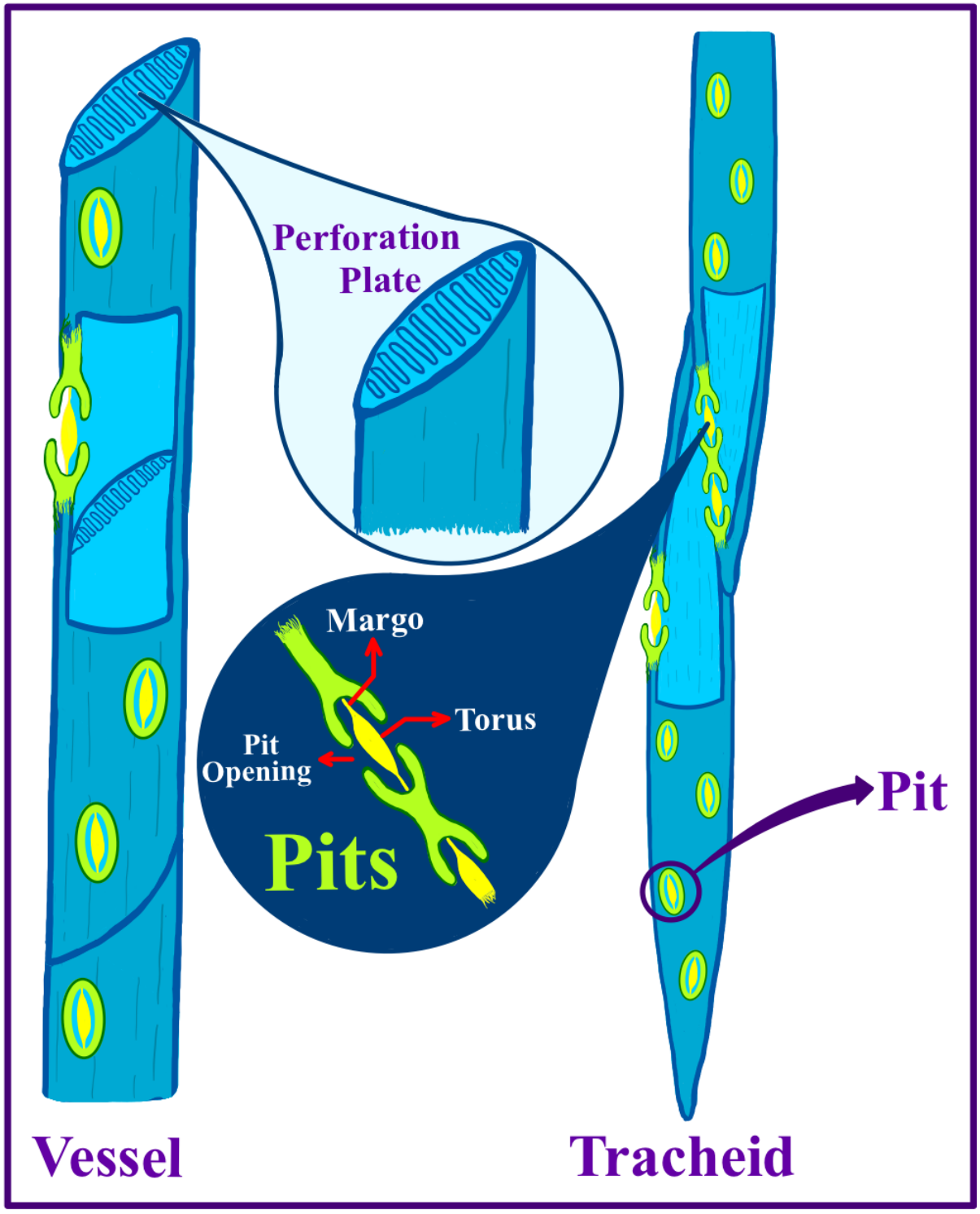

Figure 2.2. Two types of tracheary elements

Xylem is not the only choice for pathogens to invade plants and colonize; phloem tissues can also provide a convenient route for several types of pathogens. The following section will delve into the process of phloem photosynthetic sugar transportation and its connection to microbial interactions.

2.3.2. Phloem

Phloem has source cells, sink cells, and sieve tubes (Figure 2.3). Source cells are capable of performing photosynthesis, and the photosynthetic products produced by these cells are called phloem elements. The entire phloem elements are deposited in sink cells, and sieve tubes play a role in transporting photosynthetic products from source cells to sink cells. Several factors support the transportation of photosynthetic products, including transpiration, water supply, and charged particles (ions), which create root pressure below ground. This pressure results in tension that pulls water up, causing it to move passively inside water-conducting xylem tissues due to a decreasing pressure gradient from high root pressure. Water from adjacent xylem cells is transferred osmotically into the sieve element, generating phloem pressure (hydrostatic pressure). This pressure helps sugar to move downward inside of the phloem along with the water molecules (Figure 2.3) (Koh et al. 2012; Turgeon 2010).

The downward transfer of photosynthetic sugar from source cells to the below ground part of the plant supports colonization of some pathogenic species in the root section. Photosynthetically fixed carbons are released to the rhizosphere from roots, making roots an essential sugar source for the microbial community. At the same time, the pathogen colonization in the roots triggers the passive chemical defense mechanisms in plants. These mechanisms result in the accumulation of antimicrobial compounds in the roots. As a result, a significant nutrient pool forms, consisting of cellular components (cell walls, cell membranes, etc.) from dead, sloughed, and decaying root tissues and pathogens (Broeckling et al. 2008).

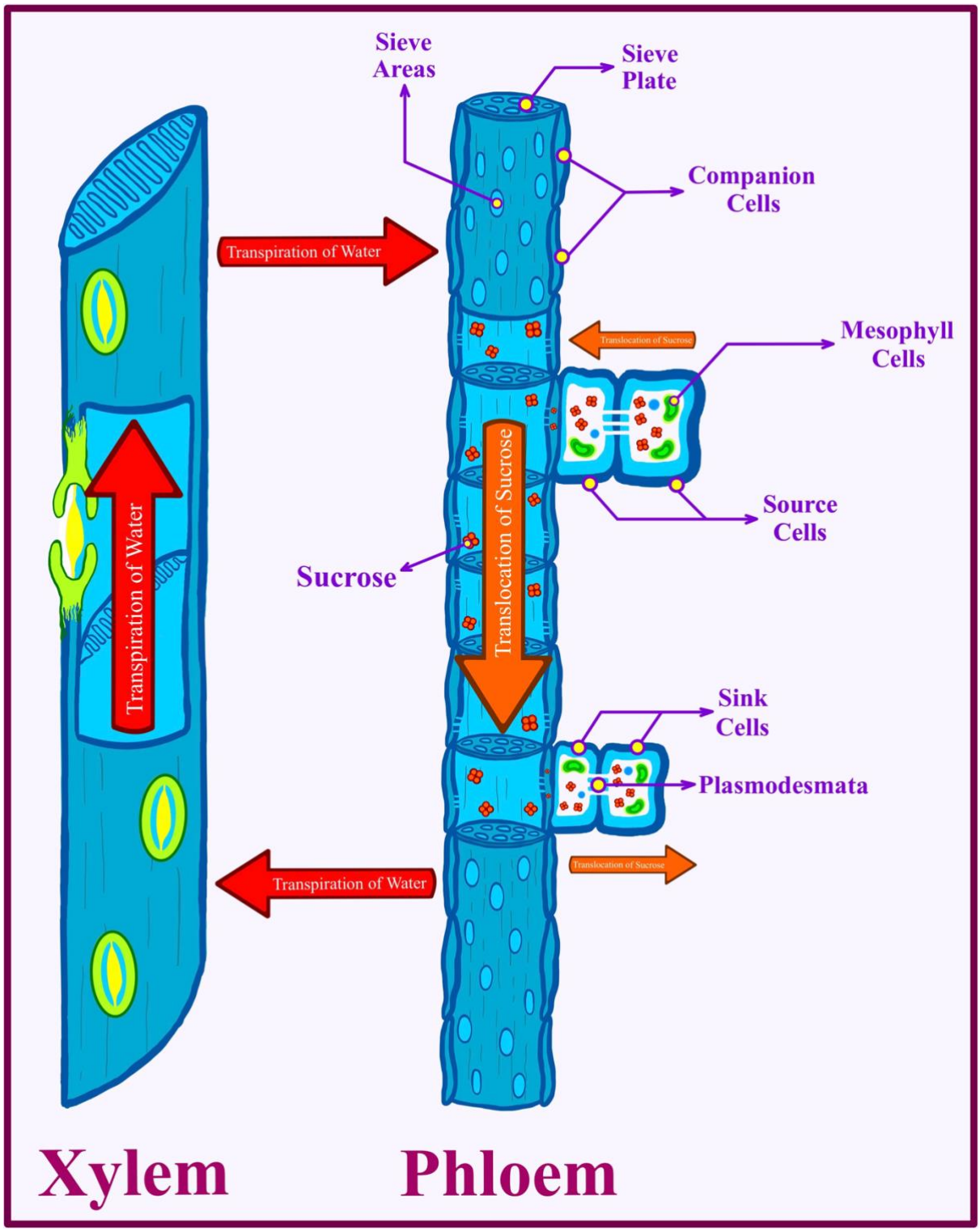

Figure 2.3. Downward transportation of photosynthetic sugar inside the phloem

The following sections will explain the cuticle and its various components, as well as their roles in plant defense against pathogens.

2.4. Cuticle

The cuticle is composed of fatty acids that are covered with pectin, wax and cutin layers (Figure 2.4) (Freeman et al. 2008). The cuticle is not only the first layer that directly interacts with pathogens, but also the first barrier that prevents pathogen interaction with the plant epidermis (Epstein et al. 2006).

The thickness of the cuticle determines its water permeability. Aquatic plants have thinner cuticle layers than arid plants. Thicker cuticles are found in arid habitat plants such as xerophytes (including aeonium, agave, aloe, graptopetalum hybrids, cactus varieties and other succulents), and they play a vital role in preventing loss of water (Praveen et al. 1997).

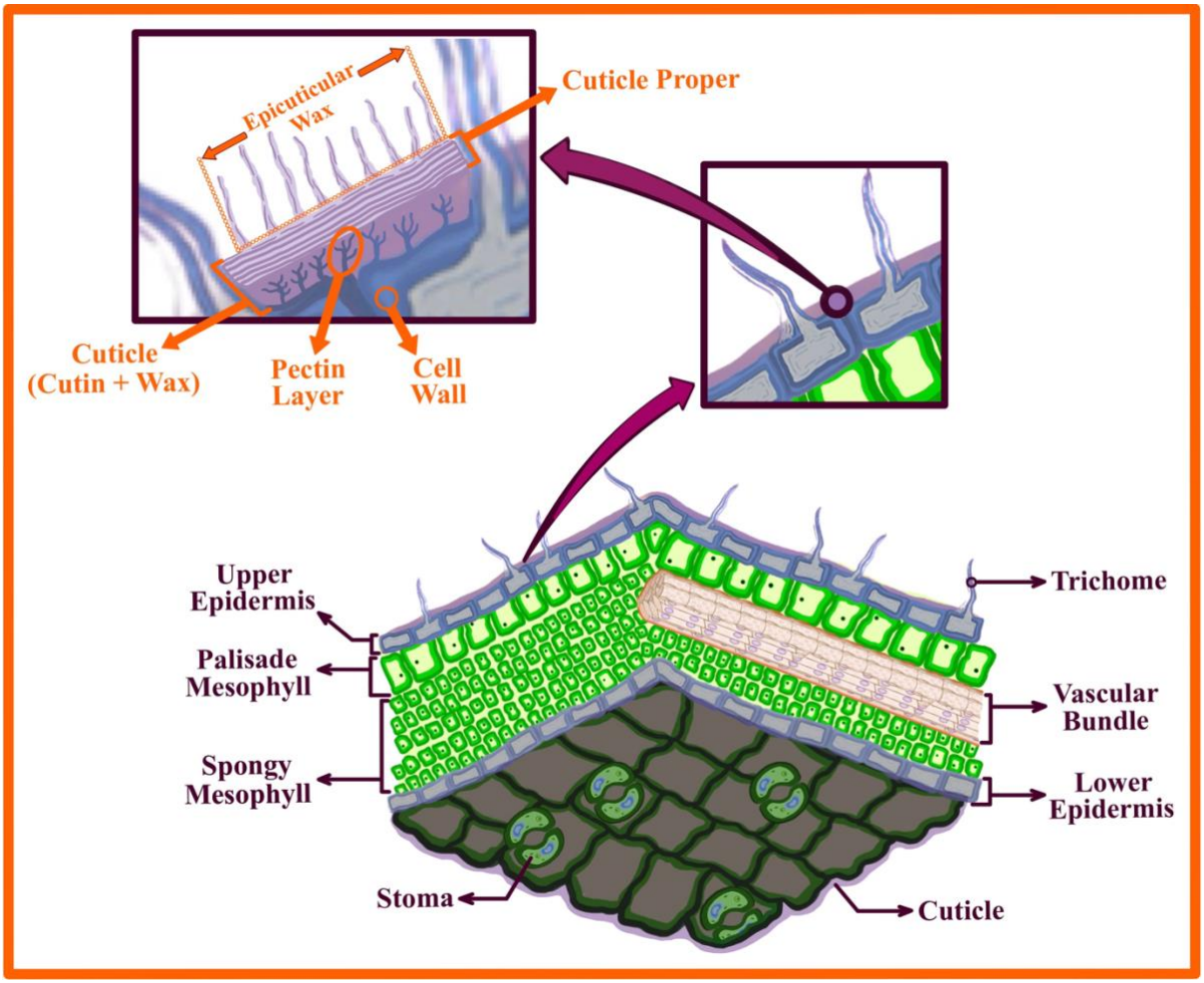

Figure 2.4. Schematized layers of the cuticle

2.4.1. Wax layer

Many fungal pathogens require water to adhere to leaves for invasion. Due to the hydrophobic characteristics of the waxy cuticle layer, which is composed of aliphatic compounds and fatty acids, the adhesion of water molecules to the plant's surface is prevented. This is an essential mechanism by which the plant protects itself against the interaction of pathogens with the leaf surface and spore germination (Figure 2.4) (Praveen et al. 1997).

2.4.2. Pectin

Pectin is a galacturonic acid-rich polysaccharide, including homogalacturonan, rhamnogalacturonan-I, and rhamnogalacturonan-II, that is found in the pectic cuticle layer (Figure 2.4). This intracellular layer act as a structural fiber that holds the cuticle layers together. Pectin is not only found in the cuticle but also in the primary cell wall of plant cells. The pectic matrix, a hydrated gel-like structure, holds the cell wall in one piece, and is produced by the activity of pectin-modifying enzymes and the endomembrane system (Van et al. 2007; Willats et al. 2001).

2.4.3. Cutin

Cutin is a biopolyester that has inter-esterified epoxy-hydroxy fatty acid chains and inter-esterified hydroxy fatty acid chains, each composed of 16-18 carbons. These fatty acid chains can be present in both the secondary cell wall and the primary cell wall. They can also be deposited in plant body tissues, such as bark. Cutin polymers form a complex mixture within the layers of waxes, including intracuticular and epicuticular waxes (Figure 2.4). Additionally, cutin polymers interact with pectin molecules, wax molecules, and other long fatty acid chain derivatives (Nawrath 2002). The fatty acid composition of the cuticle also generates a negative charge on the surface of leaves, which repels airborne pathogens such as microbes, spores, and propagules (Praveen et al. 1997).

2.4.4. Cuticle-Degrading Enzymes

Cuticle-degrading enzymes are secreted by pathogens to enter the plant tissues. The enzymes that can be listed in this category include cutinase and pectinase (hydrolases, pectin esterase, and lyases).

Cutinase is a degradation enzyme secreted by certain pathogenic species. This enzyme reacts with the cutin layer of the cuticle and catalyzes the perforation of the plant epidermis (Epstein

et al., 2006). Cutinolytic enzymes, such as aprolactone (PCL) depolymerase secreted by phytopathogenic *Fusarium*, exhibit hydrolytic activity and participate in esterification and transesterification reactions (Kim et al., 2006).

Based on their function, the three types of pectinases are: hydrolases, pectin esterases, and lyases. Pectin esterase is responsible for catalyzing the de-esterification of the methoxyl group of pectin, resulting in the production of pectic acid. The α-1,4-glycosidic linkage of pectic acid and pectin is hydrolytically cleaved by *hydrolyses* (such as polygalacturonases and polymethylgalacturonases) and is cleaved by a trans-elimination reaction catalyzed by *lyases* (polygalacturonate lyase and lyase). Unsaturated galacturonates and methyl galacturonates are formed as products at the end of the trans-elimination reaction (Garg et al., 2016).

The following sections will present an overview of the plant epidermis. The significance of the epidermis, silicon, and suberin and their roles in protecting plants against pathogens will be discussed.

2.5. Epidermal layer (Epidermis)

The plant epidermis is a layer that has many different types of cells. The epidermis is found below the cuticle and surrounds the palisade parenchyma layer, which has single-layered photosynthetic cells. The epidermal layer covers primary growth such as floral parts, seeds, fruits, stems, roots, and leaves of plants, providing protection against pathogens (Figure 2.5). It serves as the second barrier after the cuticle (Xu et al., 1996; Graça, 2015). While the epidermis is typically formed by a single layer of epidermal cells, some plant species have multiple epidermis layers, which are also referred to as ‘window tissues’ (Franceschi 2001; Doughari 2015).

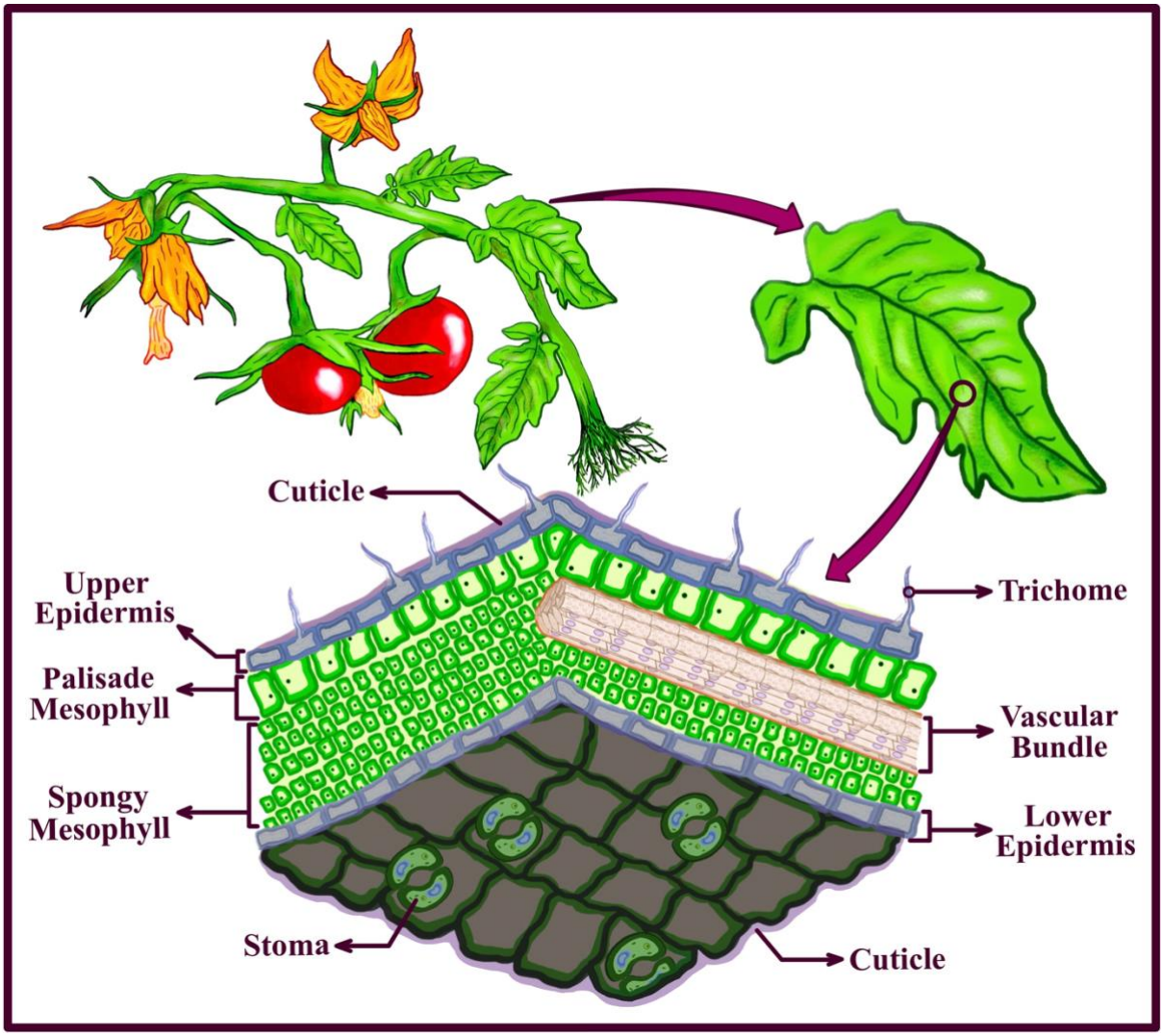

Figure 2.5. Cross-section of a leaf

2.5.1. Silicon

Silicon (Si), as an inorganic compound, accumulates in epidermal cell walls and blocks the pathway of fungal hyphae. Silicification is a crucial process for plants to protect themselves against herbivore attacks and grazing animals. Various silicified plant species cause tooth erosion in grazing animals. Silicon not only plays a significant role in passive plant immunity but also contributes to the strength of plant cell structure. Silicon and silicified specialized cells, such as bulliform cells or silica cells, are found in various parts of plants, including leaves, roots, panicles, aleurone layers of caryopses, fruit exocarps, outer cell walls of papillae, prickle hairs of glumes, epidermis of lemma, trichomes, and trichome-like structures, such as leaf micro-hairs (Kumar et al., 2017a; Graça 2015). Section 2.13.4 will provide more information about the importance of silicon in plants.

2.5.2. Suberin

Suberin is a unique lipophilic macromolecule composed of glycerol and aliphatic-phenolic suberin acids (multi-functional long-chain fatty acids) with its polyphenolic and polyaliphatic domains. This biopolymer is found in the structures of the periderm, root epidermis, and secondary cell wall. Suberization is the process of accumulating suberin biopolyesters around plant cells. This process inhibits the loss of water in healthy cells, reduces the possibility of pathogenic infections in damaged tissues by isolating wounds, and dampens the invasive activity of pathogens by enclosing the infected tissues (Graça 2015).

2.6. Periderm

Periderm is a specialized form of the epidermis that serves as a mechanical barrier, similar to the cuticle and epidermis. It has multicellular layers. Plants with secondary growth, such as shrubs and trees, are enveloped by a periderm layer, which includes cork and cork cambium.

Suberization is a process that involves the accumulation of suberin polymers. During suberization, suberin molecules become concentrated in the epidermis. This high concentration of suberin triggers the differentiation of the epidermis, leading to the thickening of the plant's outer shell to form the periderm layers. The periderms act as a pre-invasive barrier that prevents the passage of water and the movement of pathogens through the epidermal host cells (Graça 2015; Park et al., 2004).

2.7. Callose

The plant cytoskeleton functions as a physiological barrier that is made up of actin filaments, which are responsible for vesicular transportation of antimicrobial compounds, and callose, which regulates the plant's response under conditions of biotic and abiotic stress (Park et al., 2004; Wang et al., 2005). Callose is composed of β-(1, 3)-D-glucan polymers with β-1,6-branches, and is typically deposited inside pollen and cell plates during cytokinesis. This polymer is synthesized in cases of infection, wound formation, physiological stresses, etc. Callose deposition in the plasmodesmata of infected cells prevents the spread of pathogens to adjacent cells by isolating infected cells. However, phytopathogens have evolved several ways to infect plant cells. It is assumed that callose is degraded by β-(1,3)-glucanases (Chen et al., 2009). Enzymes like these may be used by pathogens to overcome the barriers formed as a result of callose deposition during pathogenesis.

The following section will provide an overview of stomata, their function, and plant-pathogen interactions. This section also covers the multifaceted role of stomata in plant physiology and defense against pathogens.

2.8. Stomata and Transpiration

Stomata are pores in the epidermis that connect plant cells with the atmosphere, facilitating the exchange of O_2 and water vapor, which is necessary for photosynthesis and transpiration. Guard cells, which enclose stomata, play a crucial role in photosynthesis and the regulation of stomatal activity. Stomatal opening and closure (Figure 2.6) are influenced by various factors, including hormones (such as ABA), environmental conditions [such as daylight (UV), CO_2 levels, humidity, and turgor pressure], and biotic stresses (such as pathogenesis).

Stomata are responsible for optimizing the conditions of photosynthetic processes in plant cells. To achieve this, guard cells control the concentration of CO_2, water and O_2 by regulating the opening and closure of the pores via turgor and hydrostatic pressure modulation. Stomatal opening is triggered when the concentration of CO_2 in the intercellular gas spaces decreases. Conversely, a reduction in turgor pressure within the guard cell leads to stomatal closure. In arid and hot environments, guard cells may shrink to close stomata and limit water loss.

Furthermore, stomatal opening and closure can be controlled to eliminate favorable conditions for pathogenesis. For example, hormonal changes resulting from pathogen detection may prompt guard cells to close the stomatal openings to prevent phytopathogens from entering plant cells. Additionally, stomata may evolve to have smaller pore sizes to hinder the invasion process of pathogens (Jones et al., 2006; Gudesblat et al., 2009; Negi et al., 2014).

Initiation of Infection in Stomata:

Stomata may serve as suitable entry points for endophytes, but it is difficult for phytopathogens to infect a plant unless they create a biofilm, surface-attached bacterial clusters. After adapting to the stomatal surface, the pathogen's intercellular signaling system regulates the expression of density-dependent genes, which encode the main biofilm components like xanthan polysaccharide (xanthan gum). These kinds of components increase the chance of bacterial survivability and accumulation on the plant surface, which result in biofilm formation. Biofilm secretes several chemicals such as fusicoccin or coronatine (which have a similar composition as methyl jasmonate) that cause biotic stress in plants. When the assembled pathogen’s

concentration becomes sufficiently high, the chemicals secreted by pathogens may increase and disturbs stomatal function, that may causes the stoma to open (Jones et al., 2006; Gudesblat et al., 2009; Negi et al., 2014; DeZwaan et al., 1999; Clergeot et al., 2001).

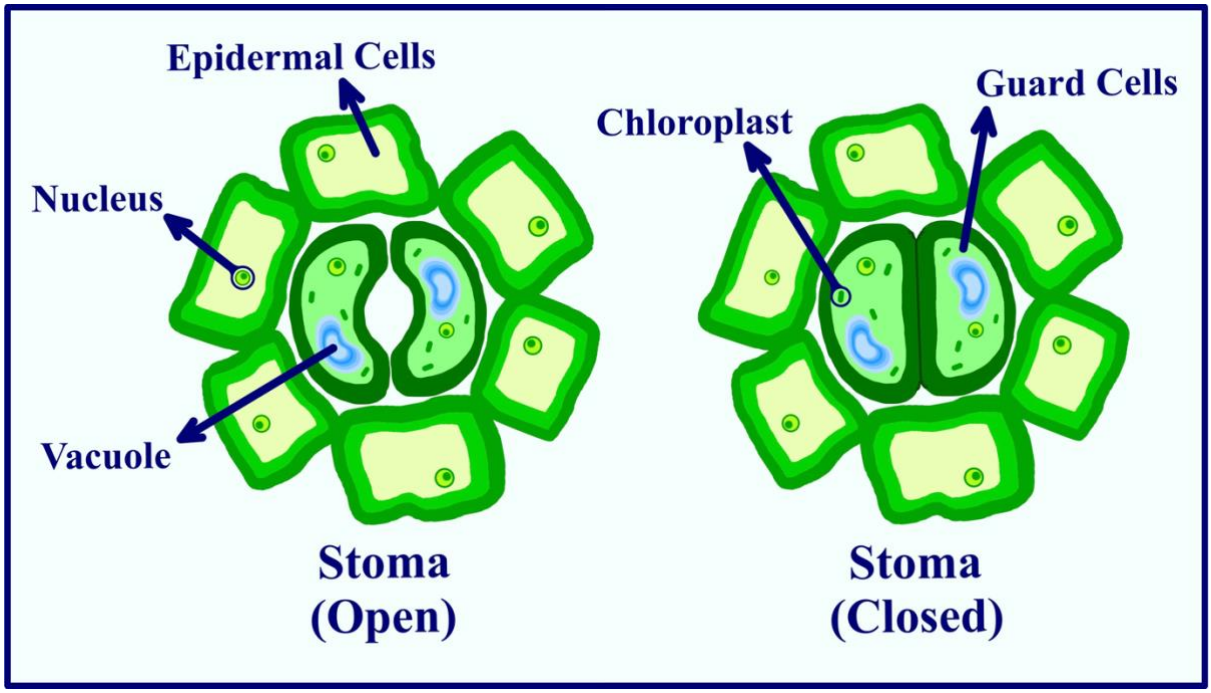

Figure 2.6. Stomatal closure and opening

2.9. Hydathodes (Water Stomata) and Guttation Process

Hydathodes, also known as water stomata, are specialized pore-like structures consisting of non-specialized living cells. They are typically found at the adaxial (upper) leaf tips, often along the margins of leaves, and serve as connections between xylem and intracellular spaces. These natural openings play a role in regulating the *guttation* process (Figure 2.7), which involves the exudation of water droplets into the atmosphere due to positive transverse osmotic xylem pressure, originating from the roots and extending to leaves, as well as the prevailing humidity level.

Guttation fluid, which is released through hydathodes, contains various minerals, including calcium (Ca), silicon (Si), phosphorus (P), magnesium (Mg), sodium (Na), and aluminum (Al). The emission or absorption of these excessive minerals through hydathodes helps to regulate the nutrient stoichiometry of plant cells (Melotto et al., 2008; Mehltreter et al., 2022). Despite their crucial role in maintaining plant cell homeostasis, these small openings also serve as entry points for pathogens to infiltrate plant cells.

Figure 2.7. Visualizing the guttation process in plant leaves

2.10. Nectarthodes

Nectarthodes are stomata-like natural pores featuring two curved guard cells that are covered with cuticle, and their primary function is to secrete sugary nectar. These openings are typically found in the epidermis and glandular tissue of open flowers, blossoms, vegetative shoot tips, and twigs. While nectarthodes secrete nectar, which disturbs sugar-intolerant pathogens, nectar can also serve as a suitable medium for certain bacterial species, such as *E. amylovor,* the

pathogen that causes fire blight disease. These bacteria can utilize nectar as a nutrient source during their life cycle, subsequently perforating entry sites and interfering with plant metabolism (Bubán et al., 2003).

The following section will provide an overview of trichomes (leaf hairs) and their multifaceted roles in plant defense and adaptation to various environmental challenges.

2.11. Trichomes (Leaf Hairs)

Trichomes are unicellular or multicellular hydrophobic epidermal cell extensions that are found above the soil in the aerial part of the plant. Thousands of these extensions, which cover the surface of the plant, function as mechanical and chemical barriers against biotic and abiotic stresses. Trichomes are differentiated based on their size (large, small, glandular), morphology, density, shape (hook, scale, head, star), and number of cells (unicellular or multicellular). Large trichomes are found on the surface of plant organs, such as leaves and stems, while smaller ones are often in the epidermis of these organs (Figure 2.8).

Trichomes play a role in pre-existing immunity, and they are specialized structures found in the epidermal surface of plant leaves. Trichomes can be glandular, or non-glandular. Some glandular trichomes are able to secrete repellents, which are composed of toxic substances, against hazards such as insects and insect eggs, pathogens, and herbivores. Furthermore, non-glandular trichomes have hook-like shapes for impaling caterpillars while they move on leaves.

Trichomes play a role in protecting plants against biotic stresses and abiotic stresses. Trichomes are a barrier against the harmful effects of ultraviolet (UV) light. These appendages act as a shield against mechanical damage that may be caused by wind and sand. For instance, these outgrowths protect the buds of some plant species, such as cotton; hence seed formation is guaranteed. Moreover, trichomes can recognize external stimulation and so change the amount of Ca^{2+} for regulating pH and triggering the active defense mechanism. Trichomes also prevent excessive transpiration by absorbing moisture and nutritional substances from the atmosphere (Wang et al., 2021).

Figure 2.8. Different types of trichomes on different plant parts

Examples of plant structures include fruits of *Vicia hybridia* (hairy yellow vetch) (a); buds of *Verbascum thapsus* (great mullein) (b); stem (c), buds (d), and leaf margins (e) of *Sonchus oleraceus* (sow thistle); margins (f) and midrib (g) of *Lactuca serriola* (prickly lettuce) leaf; sepal (j, k) and stem (m) of flower; and buds (l) of *Knautia arvensis* (field scabious); fruit (n) of *Fragaria x ananassa* (Strawberries, Strawberry); stem (r) of *Dysphania ambrosioides*

(Mexican tea), its leaf margins (o), and midrib (p); stem (s), and sepal (t) of *Daucus carota L.* (wild carrot); leaf (u) of *Cucurbita pepo* (field pumpkin); midrib (g) and stem surface (h) of several plants.

2.11.1. Stinging Nettles

Stinging nettle is a plant that has stinging hairs in its leaves, which are specialized trichomes. They may pierce the skin of herbivores and secrete a mix of some toxic chemicals, including formic acid, serotonin, acetylcholine, and histamine, which cause itching, as well as hormones like prostaglandin that stimulate pain receptors and irritate grazing animals. In high concentrations, these toxins can even be lethal to some insects (Jones et al., 2006).

2.12. Thorns, Prickles, and Spines

Thorns are rigid, elongated extensions with sharp points that provide passive protection to plants against threats like humans or animals. Thorns originate from the epidermis of stem tissues. Prickles, on the other hand, are much smaller than thorns and serve the same protective purpose. Prickles are outgrowths of epidermis and bark tissues (Figure 2.9). Spines have a similar role to thorns and prickles, but they extend from leaf tissues or vascular tissues. Cacti, for instance, are covered with spines. Glochids, a smaller type of hair-like spine, differ in their origin (Freeman et al., 2008).

Figure 2.9. Stereo microscope images of prickles and spines

Echinocereus viridiflorus (*hedgehog cactus*) (a) has prickles in its body parts, and *Lactuca serriola* (pricky lettuce) (b) has prickles on its stem; the stem of China rose (*Rosa chinensis*) (c) contains spines.

The following sections will explain idioblasts and their various subtypes, including pigmented cells, sclerenchyma, crystalliferous cells, silicified idioblasts, and long cells. It will also delve into the roles of these specialized cells in plant immunity and plant defense mechanisms.

2.13. Idioblasts

Idioblasts are immune cells that are highly differentiated and primarily protect plants with their sharp mineral crystal blades (made of calcium oxalate) on leaves. Idioblasts also store and secrete chemicals that are toxic to herbivores such as insects and mammals. Pigmented cells, sclereids, crystalliferous cells, and rows of silica cells are some of the subclasses of idioblasts.

2.13.1. Pigmented cells

Pigmented cells synthesize tannin (also defined as tannic acid) as a secondary metabolite, which is a class of poisonous gallic acid with a yellowish or brownish color. As an organic molecule, tannin is present in 5-10% of the dry mass of plant leaves and is sequestered in vacuoles. Tannins fall into two main categories: hydrolyzable (HTs) tannins, which are gallic acid and simple sugar polyester, and condensed tannins (CTs, proanthocyanidins), which are flavonoid phenol polymers. Tannin has a sharp, bitter taste, which is distasteful for many herbivores. Oxidized tannin molecules inside the gut of insects form antimicrobial quinones, semiquinone radicals, and reactive oxygen species (ROS).

Although the accumulation of ROS inside insects is poisonous, they may tolerate the toxic effects biochemically via antioxidants, surfactants, and the acidic medium that is found inside their guts. Additionally, physical protection is provided by the peritrophic envelope that lines the midgut of insects (Broderick et al., 1991; Barbehenn et al., 2011; Grebe et al., 2011; Miyashima et al., 2011).

2.13.2. Sclerenchyma

Sclerenchyma cells are classified as sclereids (stone cells) and fibers. Sclereids have thick and lignified asymmetrical secondary cell walls, which provide strength to the plant cell skeleton with their woody composition, making it hard for animals to chew plant tissue. Sclereids can be categorized morphologically as short sclereids, macrosclereids, osteosclereids, astrosclereids, and trichosclereids. These cells are found in leaves, fruits (especially the flesh of pears), seeds, and non-growing parts such as mature stems and bark. The maturation of sclerenchyma tissues ends with apoptosis, typically resulting in dead cells. The presence of

stone cells inside the flesh of pear fruit can cause dental erosion in animals that feed on them over the long term (Mazen et al., 2004; Cheng et al., 2019).

2.13.3. Crystalliferous Cells (Crystal idioblasts)

Crystalliferous cells**,** also known as crystal idioblasts, are specialized cells that contain calcium oxalate biomineral within vacuoles that are embedded in the structure of the palisade cell layer. Calcium oxalate filled vacuole formations regulate calcium levels within the plant. These crystals may also play a role in enhancing photosynthesis by dispersing light through chloroplasts or reflecting excess light to the epidermis. As a form of physical protection, crystalliferous cells can harm the mouthparts of grazing animals, potentially causing choking and swelling. These types of idioblasts are commonly found in tropical house plants like peperomia and can exhibit various crystal shapes (Franceschi 2001; Doughari 2015).

2.13.4. Silicified Idioblasts

Silicified idioblasts contain a high amount of silicon (Si) due to the osmosis that regulates evaporation and moisture uptake processes in plants through the symplastic pathway (Kumar et al., 2017a). These specialized cells provide passive innate immunity to the plant. Due to their high silicon content, these cells can damage the mouthparts of herbivores, leading to dental erosion over time. As a result, herbivores tend to avoid plants with a high concentration of silicified idioblasts.

There are two main types of idioblast silicification: passive and controlled. Passive silicification occurs without metabolic control by cells and is a result of intense transpiration leading to water loss (dehydration), which results in the deposition of Si in random cell walls (Kumar et al., 2017a). Controlled silicification involves the granular deposition of silica in the matrix of the primary cell wall. This process induces the polymerization of silicic acid and can be observed in both living and dead lumens, which are membrane-defined cavities inside the cell (Kumar et al., 2017a; Chen 2015).

2.13.5. Long Cells

Long cells, also known as fibers, are a type of sclerenchyma cell and a subclass of idioblast, with their intertwined, elongated cells found in the root, stem, and vascular bundles of leaves. They contribute to the strength of the plant’s skeleton (Kumar et al., 2017b).

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