Spring 1-1-2012

A Comprehensive Analysis and Elementary Model of Phototropism and Gravitropism

Jonathan Jorge Figueroa
University of Colorado Boulder, jonathanjfigueroa@gmail.com

A COMPREHENSIVE ANALYSIS AND ELEMENTARY MODEL OF PHOTOTROPISM AND GRAVITROPISM

A THESIS SUBMITTED TO THE

FACULTY OF THE GRADUATE SCHOOL OF

THE UNIVERSITY OF COLORADO

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF CIVIL, ENVIRONMENTAL,

AND ARCHITECTURAL ENGINEERING

Jonathan Jorge Figueroa

July 2012

A Comprehensive Analysis and Elementary Model of Phototropism and Gravitropism

by Jonathan Jorge Figueroa

The final copy of this thesis has been examined by the signatories, and we find that both the content and form meet acceptable presentation standards of scholarly work for the Department of Civil, Environmental, and Architectural Engineering

_____

(Franck Vernerey) Principal Advisor

_____

(Ronald Y.S. Pak)

_____

(Mark Stoykovich)

Abstract

Figueroa, Jonathan Jorge (M.S., Civil, Environmental, and Architectural Engineering)

A Comprehensive Analysis and Elementary Model of Phototropism and Gravitropism

Thesis directed by Assistant Professor Franck Vernerey

The utilization of the surrounding environment is a distinct feature that plants harness to sustain equilibrium and continual maintenance. Plants undergo rapid changes and various stresses that add to an already complex system consisting of elaborate functions. Phototropism involves the activation of photoreceptive pigments, cell proteins and plant hormones through light reception which brings about curvature. Gravitropism is induced through the sedimentation of starch sacs that activate cells and allows for curvature response to gravity. Both processes are vital to the plant and can result in various structural responses. Recent research points to a specific plant hormone specified as auxin as the catalyst for differential growth and curvature.

An extensive analysis reveals the exact mechanisms behind phototropism and gravitropism from the molecular level to the global level. A plant experiencing a phototropic response utilizes specific pigments that differentiate the spectra of light. These protein based pigments localize to the plasma membrane of the plant cells and induces a state of phosphorylation. More proteins are activated and begin to interact with the naturally occurring auxin. Auxin then moves laterally across the different cell layers of the plant and produces curvature. Gravitropism follows a similar pattern but it is the endodermis of the plant that recognizes gravity. There are distinct organelles within the vacuoles of the cell that sediment in

response to the vector of gravity. Upon sedimentation, an interaction with the cell's cytoskeleton takes place and proteins are again activated. Interaction with auxin occurs resulting in lateral movement that initiates curvature.

Cell expansion is the result of the interaction between auxin and the cell. Internal turgor pressure and cell wall compliance are the two main factors involved in cell expansion. A model is created with the relationship of auxin and cell expansion in terms of the different tropisms. In the model, the stem of a plant is considered and assumptions of curvature are made. Experimental data is gathered and comparisons with the model are drawn. The model is a substantial starting point but has room for improvement. The proper curvature response is simulated and potential applications are explored.

Acknowledgements

I would first like to thank my superb advisor, Professor Franck Vernerey, for his bountiful wisdom and continued support throughout my studies at the University of Colorado. This research project would not have existed without the assistance of Professor Vernerey and I am honored to have spent my time under his excellent tutelage in this prestigious graduate school. I would also like to thank the other members of my graduate committee, Professor Ronald Y.S. Pak and Professor Mark Stoykovich, for their added support, effective suggestions, and positive criticisms. Their assistance truly helped me in successfully compiling an effective thesis report that, above all, meets standard expectations.

Two years ago the concept of graduate school seemed more like a dream than a potential reality. The economy was in a rough patch and the decision to further apply myself in the world of academia seemed necessary. Upon receiving my acceptance letter, I told myself that this was a privilege that must not be wasted. I embraced the University of Colorado with loving arms and proceeded to get my Master's Degree. I have been truly blessed and looking back on the first day of graduate school I know I made the right decision.

Finally, I would like to thank my wonderful family and friends who have continuously supported me throughout my entire life. Also, I dedicate this to my loving girlfriend, Lauren, who has always been by my side in both the good and stressful times. Thank you for everything.

Contents

List of Tables

List of Figures

Chapter One

Introduction

1.1 A History of Tropisms

The continuous advancement of the world's infrastructure is an insatiable hunger that has and still is a defining characteristic of human society. This is the basis for research and brings about different avenues for modeling and experimentation. Beam analysis has been a prominent area of interest with concepts like the relevance of surface effects on nanobeams providing insights on mechanical behavior and material properties [1]. The structural response to various applied forces has produced models with efficient and precise accuracy. The next step is to model a response in which the structure utilizes the environment in which it is encompassed. Plants, which are biological structures, harness their surroundings and react to various stimuli.

The process in which plants respond to their environment has been a topic of interest for centuries. The earliest Greek philosophers believed that plants are sensitive and capable of motion [2]. However, it wasn't until the 18^{th} century that scientists began to exam the exact physiology of plants. In 1806, Thomas Knight postulated that a plant's perception of gravity may dictate the shoots to grow upwards and the roots to grow downwards [3]. The force of gravity was a vector that plants used to adjust to the environment. Albert Bernard Frank was the first to propose that phototropism and gravitropism are inductive responses sharing a common underlying process [4]. Scientists continued to push this theory and a mechanistic connection between the two processes was formulated. Charles Darwin proposed that the back and forth

nutation associated with plant growth could be directed by a stimulus such as light or gravity [5]. As the prefixes indicate, phototropism and gravitropism are processes involving light and gravity.

It was the work of Darwin that led scientists down a new path to solve the mechanistic riddle behind plant functions. The concept of circumnutation allowed Darwin to propose a relationship for phototropic and gravitropic responses. Circumnutation, or nutation as the Dictionary of Botany states, is the circular or elliptical movement of the stem tips of many plants [6]. This circular movement is analogous to a swinging motion, which seems to be dictated by both internal stimuli and gravity [6]. It was this combination of nutation and plant response that brought Darwin to a prominent discovery. He was able to discern that the site of photoreception in the shoot tip is separable from the location of curvature [2]. The site in which light is perceived is different from the location in which the plant has a curved response. With insightful observation, Darwin postulated that a transmissible substance produced in the shoot tip is responsible for inducing curvature in the lower regions of the plant [5]. The emergence of this substance brought about the discovery of the first plant hormone, auxin [2]. Auxin is a pivotal plant hormone that plays a key role in plant response and growth.

At first reaction, the reliance of plant response to some hormone was dismissed and the definitions of plant function continued to change. Experiments on various plants and sections of plants arose as evidence started to gather. In 1894, the scientist Rothert used maize coleoptiles to demonstrate that the area of most light sensitivity was located at the tips [7]. This experimentation coupled with Darwin's work yielded more plant tampering. Scientists gathered more evidence indicating a transmissible substance produced in the tip does play a role in stimuli response [2]. Plant manipulation and testing grew, which lead to the composition of hypotheses and theories about the plant hormone, auxin, used to control reactions to environmental stimuli.

For gravitropism, the starch-statolith hypothesis was and continues to be the definitive process behind gravity perception with the integration of auxin [8]. In the case of phototropism, the Cholodny-Went theory unifies the plant response to light with the movement of auxin [8, 9]. With these principles, the basis behind plant movement can be understood but a more in-depth approach is needed if a model is to be established.

The starch-statolith hypothesis asserts that organelles or statoliths, also known as amyloplasts, sediment to the bottom of gravity perceiving cells producing a chemical signal that regulates differential growth [8]. Essentially, cells within a specific area of the plant respond to the gravity vector and send a signal to promote varying growth along the sides of those cells. Gravitropic response and the starch-statolith hypothesis have been present for decades but the recent years of technological improvement have allowed for a more precise examination. The transformation of the mechanical process from gravity to the chemical signal that is associated with differential growth is vital to unlocking gravitropism. Interaction between the amyloplasts and other cell components such as the endoplasmic reticulum, cytoskeleton, or vacuole are potential possibilities [8]. Amyloplasts and their surrounding cellular environment are talking to each other to perpetuate a growth response. Thus, it is important to look at the cellular level of gravitropic curvature to distinguish the exact mechanism that translates to the plant's global level.

A relationship between auxin and phototropism is defined through the popular Cholodny-Went theory. The theory concludes that tropisms are induced by the lateral asymmetry of auxin as a result of lateral translocation [9]. It is the asymmetric movement of auxin throughout the plant that induces differential growth which causes the curved response. Phototropism utilizes light stimuli to mediate the movement of auxin from the light side to the shaded side of the plant

[2]. The presence of light redirects the flow of auxin to the region which lacks light and induces the plant to curve in the direction of light.

Despite being the accepted theory to explain phototropic curvature, there have been various challenges to debunk this concept. In 1919, A.H. Blaauw speculated that phototropism was a secondary process that came from differential growth inhibition in relation to photomorphogenesis [10]. Blaauw's theory suggested that light, not auxin, is responsible for the growth response of plants. Scientists pursued the potential idea that both theories played a part in phototropic movement. However, the work of Daniel Cosgrove and Emmanuel Liscum through plant testing produced evidence that contested Blaauw's theory. Results from experimentation illustrated that growth inhibition due to light occurred sooner than phototropic curvature and that each process functions separately [11]. The mechanics behind phototropism shifted back to the role of auxin. Another hypothesis tried to test the validity of the Cholodny-Went theory by implementing the role of phototropic light receptors. The hypothesis declared that photoreceptors called carotenoids interact with auxin to render them inactive [12]. Essentially, light shining on a particular portion of the plant would be recognized by carotenoids and in turn those photoreceptors eliminated the use for auxin in that region. But, further research found no change in overall auxin concentration. The Cholodny-Went theory has continued to withstand test after test while gaining strength along the way. From 1963 to 1964, scientists found a correlation between auxin and the magnitude of phototropic curvature as well as plant testing that resulted in the accumulation of auxin near the shaded side of the stem [2]. Information on the precise mechanisms of phototropism has grown exponentially and a constant amongst this compilation of knowledge is the role of auxin.

1.2 Motivation and Objectives

Formulation of hypotheses and past plant experimentation has shown that plant structure is important to the system as whole. It is not simply the movement of auxin throughout the system but also the network of the plant that delegates the motion of the plant hormone. To understand the responses of phototropism and gravitropism it is vital to examine the global, local, cellular and molecular levels. At the global level, the plant is considered whole and the curvature response to light or gravity is distinctly visualized. Locally, the various layers of the plant, both outside and within, are examined and important regions are distinguished. Within those layers are cells that interact with each other to respond to stimuli. Each cell consists of molecules that form the contents within that interact and initializes stimuli response. All of these levels are vital to unlocking the biological processes of phototropism and gravitropism.

Plant structure is the foundation to such biological systems and once established, the mechanisms within can be fully comprehended. Auxin is the vital plant hormone that is involved with differential growth and how it moves throughout the plant system is significant. History has provided a general outline in regards to auxin movement but a precise examination will reveal its effect from the molecular to global level. Gravitropism uses the starch-statolith hypothesis to define the process in which gravity is perceived. Amyloplasts sediment within cells but what exactly is behind this function that translates to a chemical signal? Once this chemical signal is induced, how are auxin and the associated differential growth affected? The Cholodny-Went theory has specified that differential growth involving phototropism is a result of the lateral asymmetry of auxin. But how does a plant take light stimuli and transform it into a growth response? The introduction of photoreceptors has established a possible link but the connection

to auxin is still shrouded. An exhaustive analysis will pinpoint the definitive mechanisms at the molecular and cellular level with respect to global response.

A clear and decisive breakdown of plant structure and mechanics allows for the construction of the overall reaction to environmental stimuli. Comprehension of these biological processes thus results in the ability to formulate a potential mathematical model. Models can be a double edged sword because they can be very versatile but disconnect can still arise with actual results. Thus, the processes behind phototropism and gravitropism must be spot on for there to be any chance in emulating such responses. Another avenue that must be explored is the relationship between phototropic and gravitropic curvature. As a plant is responding to light and reorienting itself in the direction of light, it must also compensate for the ubiquitous gravity vector. The mathematical model will have to take into account both curvature responses for a complete approach.

The end result for this research is to contribute a model that simulates tropic curvature and in turn open the doors for structural analysis using materials that are biologically altered. At a global scale, the world is pushing for a greener environment with institutions incorporating different techniques like LEED certification. Environmental effects have become a larger factor in design management and construction. The possibility of harnessing these environmental stimuli would have an incredible impact on structural design. The model found in this research article could have the potential to unlock the power behind environmental utilization.

1.3 Scope and Organization

In this paper, a thorough explanation of plant motion will coincide with the proper steps that must be taken to sufficiently model tropic curvature and the application to stem-beam

analysis. First, the structure of the plant is broken down to understand and visualize the exact areas of motion. A progression from the global level to the molecular level will define the outside and inside of the plant. Knowledge of the structure is combined with plant mechanics to demonstrate the processes behind differential growth. A further dissection of the starch-statolith hypothesis, the Cholodny-Went theory, and the role of auxin will be investigated. The interaction at the molecular level is examined and explains how the plant moves. Essentially, specific molecules within the cell will be defined as well as how they function throughout each plant level. With the combination of these functions, a thorough explanation of phototropism and gravitropism is formulated to illustrate the exact motion of plants. Specific elements that are vital to plant movement are defined and the role of auxin in each process is clarified. Various experiments are discussed and the rates of each process are shown. Biological alteration of plants points to specific responses and the importance of certain anatomical regions. A mathematical model is then put together incorporating phototropism and gravitropism. The importance of both processes is investigated and the parameters for the model are succinctly laid out. Results from the model will be compared with experimental data to test the validity of the model. Finally, the important findings from this research are summarized and the potential for further application will be explored.

Chapter Two

The System and Structure of Plants

2.1 Overview

Before understanding the mechanisms that provide plants with the ability to grow and react to environmental stimuli, its composition must be explored. There are numerous plant species across the globe that vary in size and growth response. However, the overall body of each and every plant can be broken down into two regions. A central axis divides the biological structure into two areas, the shoot in which a majority is above ground and the root that lies below the surface [13]. Each region plays a vital role in the development of the plant and can be further examined. As the breakdown continues, there are organs within each domain that serve a specific purpose. Further dissection shows that each of these organs is made up of tissues, which are a collection of cells that share a common physiological and structural function [13]. Within each cell are the necessary molecular components that dictate various processes for the plant. Cellular and molecular movements are the basis for plant function and growth. Together, each level paints a vivid picture that culminates with the crystal clear picture of plant structure.

2.2 The Outside of the Plant

A central axis separates the shoot and root system of the plant. The shoot is then divided into leaves and the stem, which is the axial component the leaves sprout from. A growing shoot forms new increments of stem and new leaves in an acropetal direction [13]. While the shoot is undergoing growth, the stem and leaves of the plant grow from the base and continue in the

upward direction. Similar to shoots, roots also elongate as the plant grows and matures. Growth of this nature has been reduced to a small region of cells located at the tip of the stem and the root. It is in this section that new cells are created through repeated subdivision and cell elongation [13]. The term meristem is given to these regions that involve the potential for unlimited growth and cell division [13]. Meristems are one of the most important parts of the plant network because they dictate the overall growth and size of the plant. The meristem initiates and largely controls all growth and organ formation pertinent to the plant [13]. It is the duty of the meristem to regulate elongation and the global growth of the plant.

Communication amongst the rest of the plant is another responsibility that involves the meristem. Signals are sent to and from the remainder of the plant body while the meristems themselves are simultaneously maintained as productive, organized areas [13]. Meristematic zones are essentially the boss of the growth operation while the rest of the plant can be emulated as the employees. Instructions are given out via the boss and followed by the employees, while the employees provide feedback. Without the meristem, a plant would cease to function and adaptation to the surrounding environment would be nonexistent.

The classification of a meristem region is dependent upon two factors. Topographic position and cell division capacity are what separate the meristem from the rest of the body [13]. A meristem that is constrained to the extreme tips of stems, branches, and roots are labeled as apical meristems. These areas result in primary growth and an increase along the length of the axis [13]. Both shoots and roots have apical meristems but they function differently in each case. In shoots, apical meristems contribute to the growth of organs such as leaves, branches, or floral parts while in roots there is an increase in root tissue and the number of roots [13]. Cambia, which are lateral meristems, are located as a continuous thin sheet on the periphery of the axis.

They are responsible for secondary growth that attributes to the thickness of the axis [13]. These lateral meristems are found on the sides of the shoot and are the major areas of interest for research. In Figure 2.1 the location of apical and lateral meristems can be seen. The effects of phototropism and gravitropism are found in these regions.

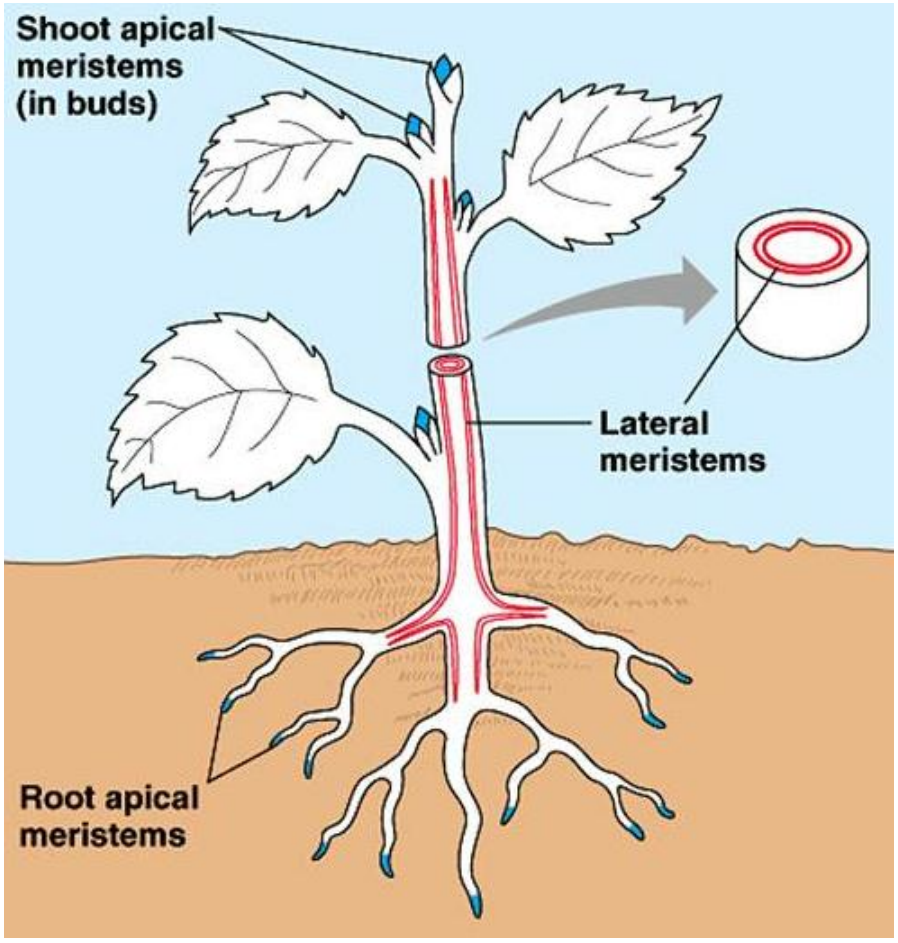

Figure 2.1 – Diagram indicating the regions in which the meristems are found both in the roots and shoots [14].

2.3 The Apex

2.3.1 The Shoot

Plant organization is a key part to how such biological structures operate and respond to different stimuli. The apex of the shoot is a sensitive area and is a major contributor to the growth process. In the shoot apical meristem there is a capacity for unlimited growth with no laterally positioned structures above the youngest leaf primordia [13]. From species to species the apex differs in size, shape, and internal structures with the involvement of age and other plant factors [13]. This is an area located at the top of the primary shoot that dictates the growth of the plant while responding to the surrounding environment.

The apex of the plant can be broken down into zones that deal with specific functions for the entire body. The initial zone contains a group of enlarged cells at the stem apex and operates as the source for all other cells of the apex as well as the entire primary shoot [13]. This central site is surrounded by the peripheral zone. The peripheral zone is derived from the central zone and creates the both the cortex and leaf primordia [13]. It is an area that regulates cells to form tissues that coalesce to establish new leaves and an integral layer within the stem. The last zone in the apex system is the transition zone. Located at the base of the apical meristem, the transition zone is formed directly from the initial zone and is an intermediate region between the initial cells and the partially differentiated cells below [13]. The transition zone produces the central pith of the stem and serves as a pathway for the transmission of hormone mediated signals to the rest of the plant [13]. Each of these locations are key to apex functionality since they control flow throughout the plant system.

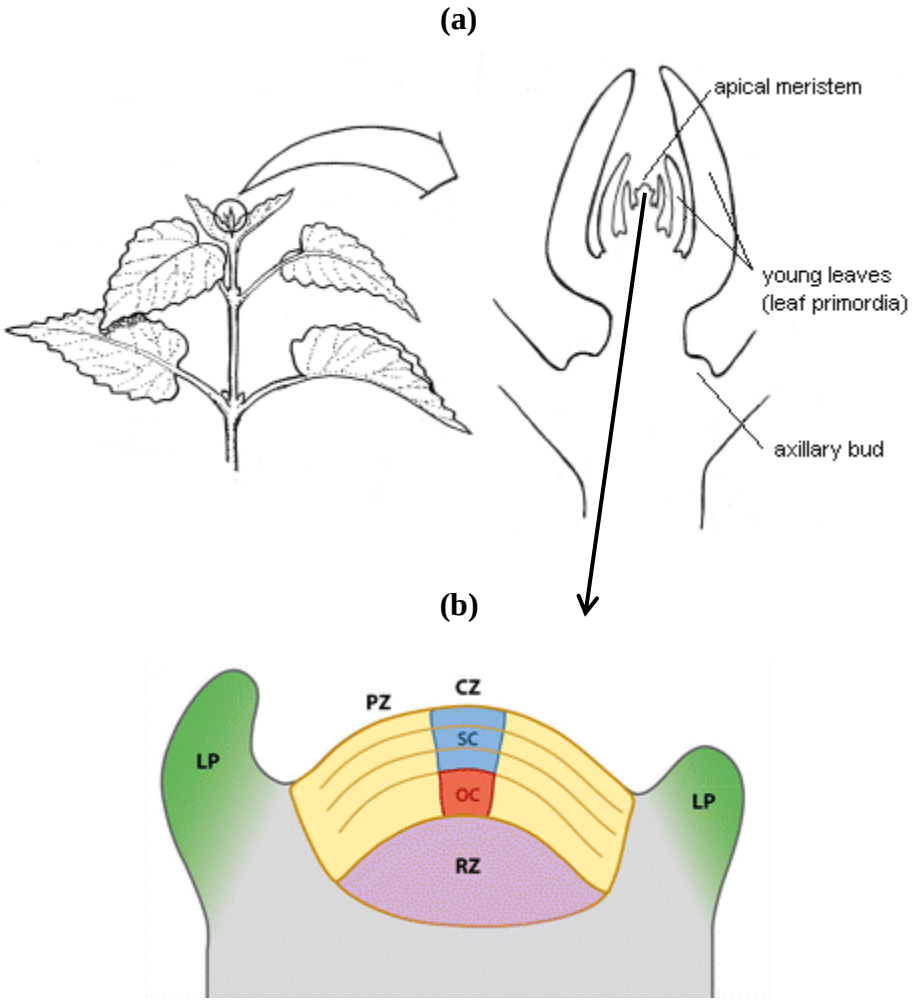

Figure 2.2 – Part (a) shows the apex of the primary shoot and zooms in to differentiate the various areas [15]. Further examination in (b) breaks down the apex into the essential zones [16]. Abbreviations: CZ, central zone also known as the initial zone; PZ, the peripheral zone; RZ, rib zone also known as the transition zone; LP, leaf primordial .

2.3.2 The Root

Similarities can be found between the shoots and roots of the plant system but there are clear differences that separate the two. Unlike the shoot, the tip of the root is covered by parenchymatous cells that form the root cap [13]. The root cap acts as a protective sheath that provides cover for the root apical meristem. The root apex can be divided into three regions and is located at the root tip. The meristematic zone is the region in which cell division occurs and is

found near the root cap [13]. From there the elongation zone continues into the root away from the root apex. In this area cells divide less and the rate of expansion growth increases [13]. A transition zone exists between the two regions and is defined at the differentiation zone. In this zone young and less differentiated cells can be located near the root apex while older and mature cells are found further away [13]. Roots are a pivotal component to plants and the apex plays a major role in root growth.

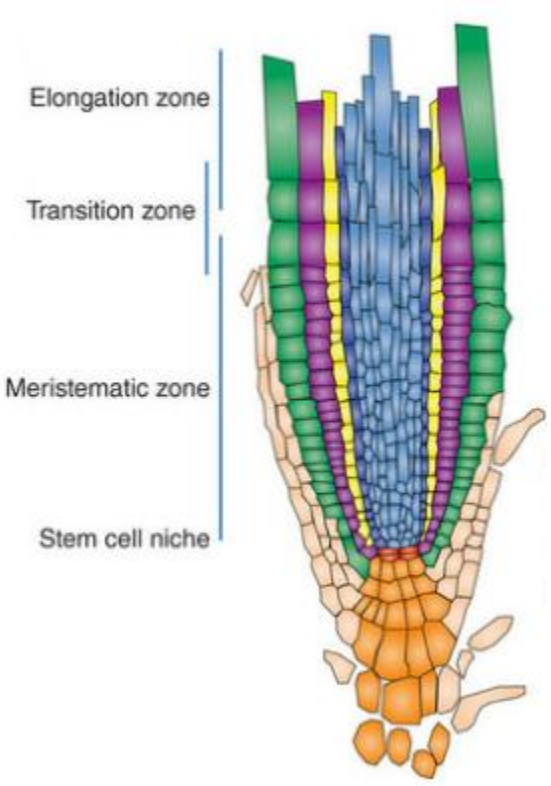

Figure 2.3 – The following depicts the various zones that are found in and around the root apex of a plant [66].

2.4 Inside the Plant

2.4.1 Overview

Identification of the outer elements involved in the makeup of a plant has rendered specific points that are necessary for growth and response. From these locations, an inside

approach will illustrate the layers involved and how they interact with one another. The composition of the shoots and roots are similar but there are differences to each component.

2.4.2 The Shoot

The outer most layer of the shoot is the epidermis. The composition of the epidermis consists of mature, uniseriate surface layer with various types of cells [13]. This layer is responsible for reacting to the environment and providing protection. The structure and chemical composition of epidermal cells function as a protective boundary layer between the environment and internal plant tissues [13]. From the epidermis the layers continue inward until the center of the plant is reached. The cortex is the next defining layer once the epidermis has been passed. Within the cortex are parenchyma cells and intracellular spaces [13]. It is the inner-most layer of the cortex that has been declared the endodermis and it is this special layer that contains relatively stable amounts of starch [13]. The endodermis will prove to be a pivotal layer in the discussion of gravitropism. The central core of the shoot is known as the pith. Certain plant species have a pith that remains as a living tissue while in others it breaks down and turns into a cavity [13]. The pith is encompassed by two tissue layers that are responsible for the transportation of various materials throughout the plant system. First is the xylem, which moves large quantities of water, organic, and inorganic solutes from the roots to the shoots [13]. Outside the xylem is the phloem, which serves as a vascular channel for the movement of organic food solutes throughout the plant [13]. Together, the pith, xylem, and phloem are the vasculature cavities that are also known as the stele [13]. Without the stele, a plant would not be able to function properly as the intake of water and important nutrients would be nonexistent.

(a) **(b)**

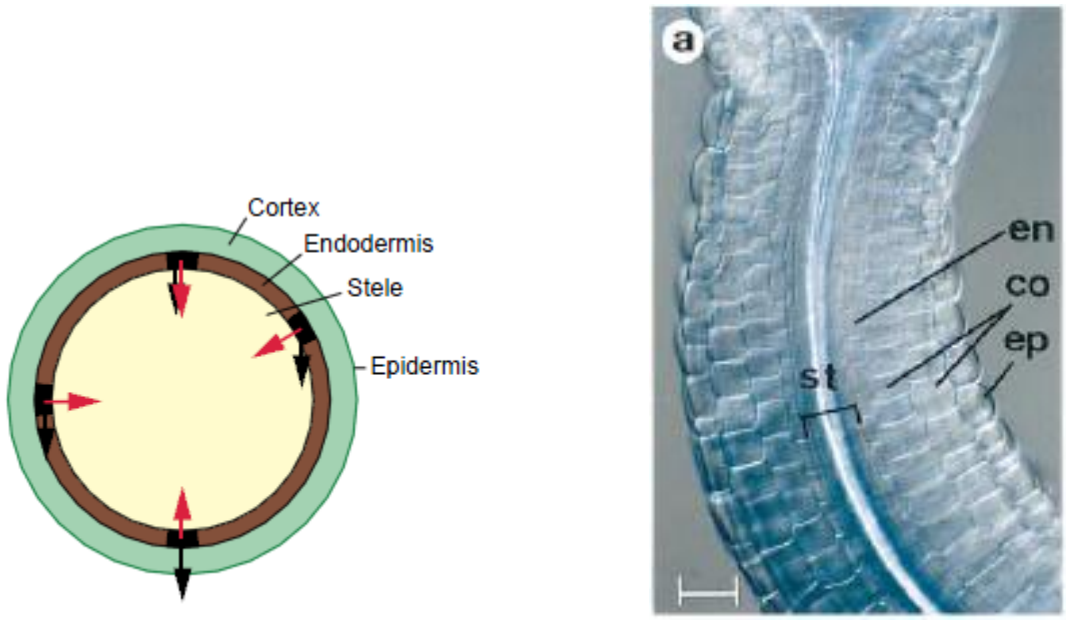

Figure 2.4 – In (a) the stem of an *Arabidopsis* plant is horizontally oriented with each of the important layers defined while (b) is a longitudinal optical section of a vertically oriented *Arabidopsis thaliana* light-grown seedling [44, 45]. Scale bar = 50μm.

2.4.3 The Root

Roots are responsible for the uptake of water and minerals found within the environment below the surface. The root system is a collective group of roots produced in the soil by a plant. Factors such as depth, degree of branching and root type differ from species to species [13]. The apex of the root is covered by the root cap and once beyond that the layers within are similar to the shoot. The first layer of the root is the epidermis followed by a thick cortex and the stele, which is defined as the inner core [13]. At the deepest layer of the cortex, the endodermis can be found and just like the shoot it contains starch grains. Interaction between the root and shoot is important for plant function. In Figure 2.1, the lateral meristem regions of the shoot and root are connected. Therefore, the layout of layers within both shoots and roots must be similar to provide a clear link between the two systems.

2.5 A Cellular and Molecular Look of Each Layer

2.5.1 The Epidermis

The layer known as the epidermis is the outermost layer protecting both shoots and roots. Within this layer are a variety of cell types that vary in shape and cell wall characteristics. The epidermis is comprised of epidermal cells, guard cells, subsidiary cells, trichomes, and various idioblasts [13]. Epidermal cells are the main cells that function as a safeguard between the environment and the innards of the plant body. They retain an active protoplast, which means that the protoplasm and cell membrane remain while the cell wall does not [13]. In addition, epidermal cells also contain a fatty substance cutin, complex organic molecules that are called lignin, silica, and waxes [13]. Guard cells are responsible for the opening and closing of the stomata, which are distinct openings within the epidermis. Such cells use turgor pressure to regulate the rate of transpiration and gaseous exchange with the atmosphere and spaces of air within the plant [13]. Control of turgor pressure allows for the expansion of the guard cells in the longitudinal direction. Subsidiary cells surround the guard cells and are involved with the storage of voluminous amounts of water and ions [13]. The epidermis is the gateway to the layers within the plant and provides protection against the environment.

2.5.2 The Cortex and Pith

The tissues that constitute the cortex and pith are composed of three principal cell types that are derived from the lateral meristem. Parenchyma, collenchyma and sclerenchyma cells are the main cell types that define the tissues of the cortex and pith [13]. Each cell type plays an important role in the axial strength of the stem. Parenchyma cells have few distinctive structural characteristics and erect the metabolic system within plants [13]. They are in control of plant

sustainability since such cells are the foundation in which all other specialized cells are formed. The size and shape of the parenchyma cell is dependent upon the internal turgor pressure and are separated by abundant intercellular space [13]. Although this cell type is known to have thin walls, at full water intake capacity parenchyma have the ability to add a decent amount of stiffness to stems and to resist local buckling [13]. The pressure due to water intake allows for an increase in axial strength while the cell itself helps in prolonging plant life.

Collenchyma cells are classified as living cells that are axially elongated and contain thickened primary walls with high tensile strength [13]. These cells offer more structural stability for the stem. Collenchyma can often be located immediately below the epidermis layer with various orientations [13]. The cell walls of collenchyma contain pectin, a complex polysaccharide, with an abundant water content [13]. Sclerenchyma are the last set of cells found within the cortex and pith regions. Similar to epidermal cells, sclerenchyma take part in protecting the plant as well as supporting it. Sclerenchyma fibers are flexible enough to bend under the force of tension and have the power to withstand a compressive force [13]. Resistance to such forces is due to the thick cell wall. The walls of sclerenchyma fibers are typically the thickest and positioned near the perimeter to counteract deformation due to bending [13]. Sclerenchyma cells are another vital part to stem stiffness and reaction to curvature.

2.5.3 The Endodermis

As previously stated, the endodermis is located at the innermost layer of the cortex. Endodermal cells are distinguished by their cell walls and the substance found in it. Suberin, a diffusion-proof substance, is located at the radial and transverse portions of the cell wall [13]. The Casparian strip is the term used to define this region of cell wall within the endodermis.

Suberin and the formation of the Casparian strip inhibit the passage of water through the radial walls [13]. The combination of the Casparian strip and there being no intercellular spaces between the endodermal cells results in substances within the stele moving through the tangential cell walls as well as the living protoplasts [13]. Another distinction within the endodermis is the presence of starch located in the starch sheath. In the starch sheath are amyloplasts that sediment, which indicates the ability of endodermal cells to sense gravity [13]. The endodermis is crucial to the concept of gravitropism.

2.5.4 The Vascular System

The core layer within the plant body is the stele, which consists of the phloem, xylem, and the pith. Similar to the cortex, the pith contains the parenchyma, collenchyma, and sclerenchyma cell types. The layers that are important here are the phloem and the xylem. Phloem is a conducting tissue that consists of different cell types. Parenchyma cells detected in the phloem function as storage cells and are associated with the sieve elements [13]. Sieve elements can be broken down into two parts. Sieve tube elements represent a highly specialized cell type that was an adaptation in flowering plants to create faster movement of organic materials [13]. Sieve cells are a less specialized group and reduced to lower vascular plants [13]. The composition of the xylem is similar to that of the phloem in that there is a diverse collection of cells. There are four cell types that that are present within the xylem: parenchyma, sclerenchyma fibers, tracheids, and vessel elements [13]. Together, tracheids and vessel elements are defined as tracheary elements and contain rigid pits in the secondary cell wall [13]. These elements are responsible for lateral conduction and support throughout the rest of the stem layers [13]. The xylem and phloem are essential to vascular movement and interaction within the stele.

2.5.5 Cellular Movement

Along with the vascular system found in plants, another transportation system is found within each living plant cell. The vacuole is a fluid-filled spaced contained by the cytoplasm of every cell in plant systems [13]. The vacuolar system is vital to plant functionality and is the connection between each cell. They are a distinct feature of plant cells and account for as much as 99% of volume within the cell [13]. Vacuoles are a necessary part of cell to cell interaction and are involved with major plant functions. Tonoplast is the single membrane that bounds the vacuole and during transport it plays a role in establishing internal turgor pressure [13]. Uptake of water is important for the cell and it is the responsibility of the vacuole to move such fluids. Besides water, vacuoles also have the ability to transport starch and protein members across the tonoplast to other cells [13]. This intercellular activity allows for the movement of important fluids and hormones, like water and auxin. The combination of the vascular system and the vacuolar system assists plants in the overall maintenance and equilibrium of the structure.

Chapter Three

Plant Mechanisms: The Role of Auxin

3.1 Overview

Plant growth and development are integral processes for overall plant function that are controlled by various hormones. One hormone that has had major implications in plant physiology is auxin. It was Charles Darwin who proposed the idea of this transmissible substance and its role in plant motion. Recent research states that auxin has been found to impact embryogenesis, vascular differentiation, organogenesis, tropic growth, and root and shoot architecture [17]. This vital hormone is an essential piece to understanding how such biological structures react and respond. The coordination between auxin and the plant system can be broken down to the molecular level. In the 1980's, auxin was linked to the regulation of gene transcription thereby establishing a relationship between the auxin signal and the expression of various genes [18]. The way in which auxin moves and the precise function that it has from the global level to the molecular level will be further discussed.

3.2 Auxin Movement

3.2.1 Polar Transport

Indole-3-acetic acid, or IAA, is the explicit chemical form of auxin that was identified in the 1930's [19]. This plant hormone has a distinct movement pattern throughout the biological structure. In the shoot the naturally occurring auxin moves unidirectionally from the apex to the base [20]. Auxin moves downward, cell to cell, from the top of the shoot to the base of the plant

structure. As IAA continues to move towards the base it reaches the central axis enters the roots of the plant. There are two distinct polarities once auxin has entered the roots: acropetally and basipetally [20]. The term acropetally is used to define the motion in which IAA moves from the central axis to the apex of the root. A basipetal motion indicates auxin being distributed from the root apex outwards laterally and back towards the base. A simple diagram shows the polar movement of auxin in Figure 3.1.

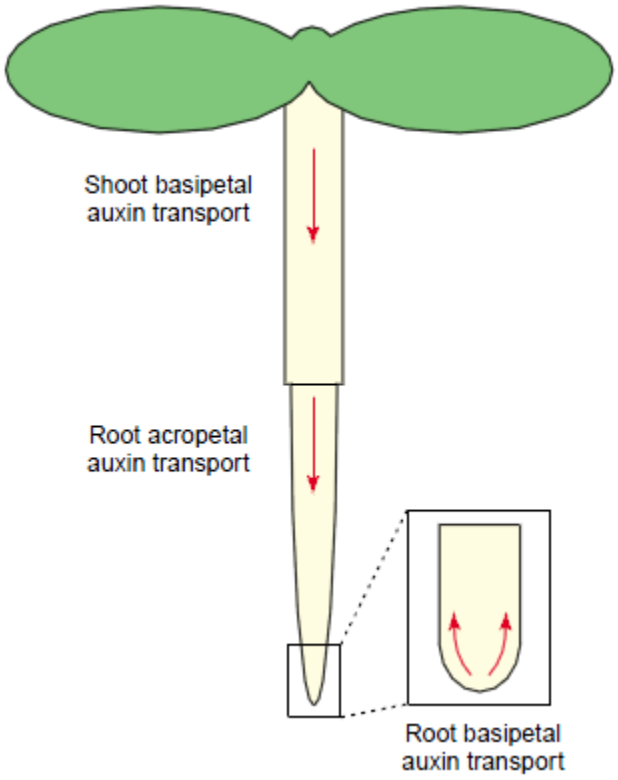

Figure 3.1 – The polar movement of auxin from the shoot apex to the root apex is illustrated with a simple plant structure [21].

3.2.2 Cellular Transport

The general motion of auxin is from the shoot apex downwards towards the root apex but the mechanisms behind the cell to cell flow must be examined. Biochemical and genetic research have distinguished a number of proteins that are involved with the cell to cell movement of auxin

[22]. Certain proteins found within the plant system act as couriers that transport IAA both in and out of the cell. Auxin enters the cell via influx carriers and exits the cell through efflux carries [21]. These carriers have certain characteristics that allow for the interaction with IAA. The AUX1 gene is a primary protein influx carrier while PIN gene family has been labeled as an efflux carrier [22, 23]. In addition to efflux transporter, there is a protein that acts as an inhibitor for auxin movement. Naphthylphthalamic-acid, NPA, is a second protein component that inhibits the transport of auxin [22, 23]. It essentially does not allow IAA to bind with the cell and accentuates the activity of the efflux carrier. As auxin enters the cell via polar transport, the combination of the efflux carrier and NPA binding site continues the movement from one cell to the next. Experimental research has shown that NPA is a peripheral membrane protein that interacts with the cytoplasm and is distinguishable from the efflux carrier [24]. A simplistic blueprint of a cell and the motion of IAA is depicted in Figure 3.2.

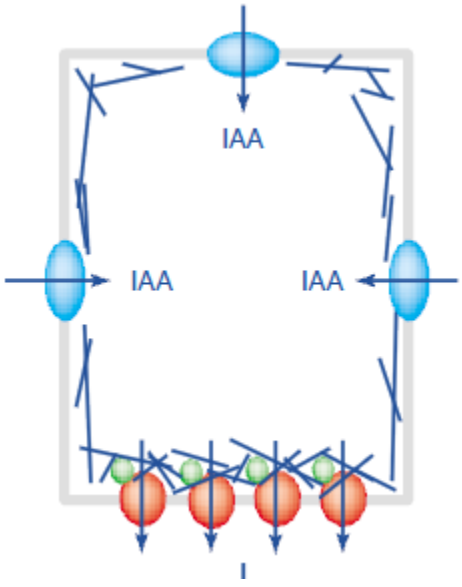

Figure 3.2 – An elementary representation of a cell and the movement of auxin in and out of the cell is depicted. The blue orbs are auxin influx carries while the red orbs are auxin efflux carriers. The rods are actin filaments while the smallest circles are NPA binding sites [21].

3.3 The Cellular Interaction with Auxin

3.3.1 Cell Division

The growth and development of plants is dependent upon specific cellular action and how auxin controls such mechanisms. Cell division, cell expansion, and cell differentiation are processes that function together to promote plant development and growth [22]. Plant development involves the creation of key plant elements. It is a continuous process that starts with embryogenesis with the formation of the primary plant body and follows with the regular generation of new roots, leaves, branches, and flowers [22]. New cells are created through cell division which leads to plant assembly. Auxin promotes cell division and the maintenance of the meristem with a vital role in cellular patterning [22]. The movement of IAA throughout the plant system is an important component to development.

First, the concept of cell division must be understood before the inclusion of auxin and its precise effects can be examined. Mitotic cell division incorporates a progression that leads to the production of two daughter cells [22]. Essentially, a cell splits in two and the two newer cells carry the same characteristics as the original cell. Therefore, as cells divide, the number of new cells increases and new components of the plant structure are formed. The cell cycle can be broken into four phases: G1, S, G2, and M. In the first phase (G1), the cell grows and once completed the (S) phase begins with the replication of DNA [22]. The cell continues to grow in the G2 phase and finally splits into two daughter cells in the (M) or mitotic phase [22]. Once this point is reached, the cycle reboots and the procedure is repeated.

Regulation of the cell-cycle is dependent upon a certain set of proteins within the cell. Distinct combinations of cyclin-dependent kinase (CDK) and cyclin complexes control cell growth during either G1 or G2 transition periods [22]. Plant manipulation has demonstrated that

CDK interacts with auxin and in turn affects the cell cycle. In Arabidopsis plants auxin was found to induce the CDK gene expression and further encoding it throughout the cell cycle [22]. Auxin acts as a catalyst to start the cell cycle and promotes cellular growth.

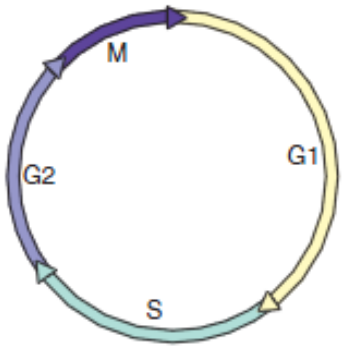

Figure 3.3 – The cell cycle is an important piece to the development of plants and is affected by the plant hormone auxin. In gap phase 1 (G1), cell growth is induced via IAA follows by DNA replication in the synthesis phase (S). The transition into gap phase 2 (G2) allows for more cellular growth and finally the cell is spilt into two daughter cells within the mitotic phase (M) [22].

3.3.2 Cell Expansion

Overall plant growth is associated with the elongation of cells within the meristems of the biological structure. The growth that plants experience corresponds to the increase in size of various components that are a result prominently from cell expansion [22]. There are two distinct processes that contribute to the increase of cell size: an increase in cell ploidy and cell expansion. Cell ploidy increases within the cell via the process of endoreplication, which is the successive replication of DNA with the absence of mitosis [22]. Cell expansion is a complex process that is regulated by internal turgor pressure and the limits of cell wall extension [22]. The introduction of auxin to these cell functions facilitates a certain reaction that is pertinent to the growth of the plant.

Similar to cell division, auxin interacts with the cell cycle and regulates certain portions. Auxin plays a major role in controlling the balance between proliferation and endoreplication [25]. Cell proliferation deals with the cell cycle mentioned above with cell division and involves the mitotic phase. Endoreplication is comprised of DNA synthesis without the splitting of the original cell. It is auxin's job to establish a balance between the two as well as regulate the functions associated with both. Research has shown that specific inhibitors of IAA have controlled the precise auxin signaling pathway in the transition from proliferation to endoreplication [22]. Figure 3.4 illustrates the cell cycle and the difference between the two processes of proliferation and endoreplication.

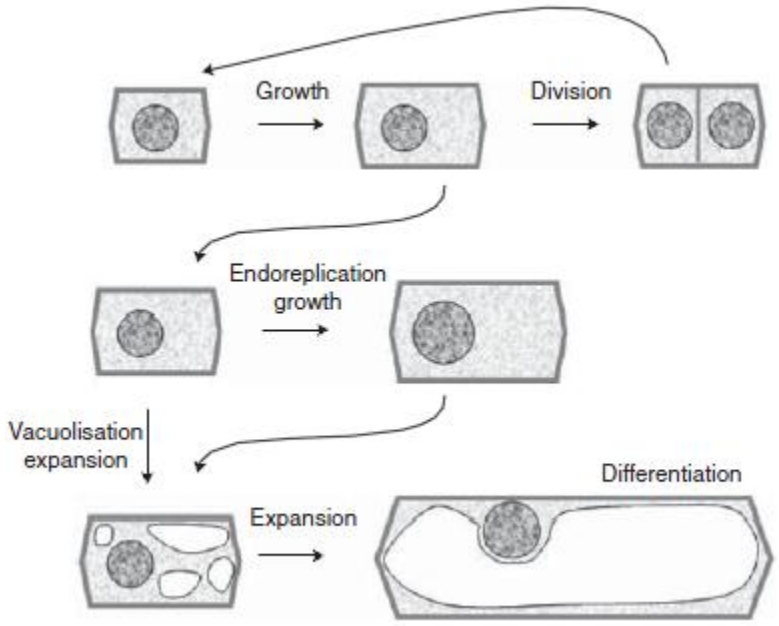

Figure 3.4 – The first line indicates the cell cycle that is affiliated with cell division. The following lines show where the cell cycle changes from proliferation to endoreplication. It is this point in cellular activity that is regulated by auxin and kept in balance [22].

The mechanism behind cell expansion and its interaction with auxin deals with a more complex process. Plant cell expansion requires the uptake of water that is stored in vacuoles and irreversible extension of the cell wall via wall stiffness reduction and the addition of new

material [22]. The vascular layers within a plant are extremely vital since survival without them cannot occur. A dynamic and intricate cell wall is the key to defining the size and shape of the cell. It is composed of a network of crystalline cellulose microfibrils interconnected with hemicelluloses and is immersed in a matrix of pectins as well as a small amount of wall proteins [26]. The cell wall is a complex layer that is dependent on molecular interactions to either stiffen or relax its overall structure. Rapid cell enlargement requires molecular changes within the cell wall network, which results in the reduction of stiffness [22]. Expansins have been distinguished as the major wall-loosening protein that are activated through acidification, which disrupts the noncovalent binding between cellulose and hemicelluloses [27]. These expansins weaken the cell wall by breaking the bonds between crystalline microfibrils and allow for expansion.

The occurrence of acidification is a direct result of auxin manipulation within the cell. Auxin activates a proton pump ATPase at the plasma membrane, inducing extrusion of hydrogen protons, extracellular acidification, and activation of expansins resulting in cell wall loosening [28]. The plasma membrane is covered by the cell wall which means that auxin enters the cell and once it reaches the plasma membrane the proton pump is activated. Once the hydrogen proton pump is activated, a hyperpolarization of the plasma membrane occurs along with the activation of voltage-dependent potassium proton channels into the cell [28]. These potassium proton channels are the passageways in which water enters the cell and promotes cell expansion [22]. In Figure 3.5, the breakdown of each cell layer and the location of the proton pumps are depicted. Together, the movement of auxin and the uptake of water are the key elements for a cell to expand.

3.4 ABP1: The Auxin Receptor

The interaction between the plant hormone auxin and the plasma membrane of the cell is the pivotal component to cell expansion. Research and experimentation have classified ABP1 as the molecular tool for auxin perception at the outer surface of the plasma membrane [29]. ABP1, or auxin binding protein, was also found to mediate auxin-induced enlargement in Arabidopsis hypocotyls [30]. This receptor interacts with IAA and initiates the activation of the proton pumps at the plasma membrane. The first ATPase pump extracts hydrogen protons while the second pump induces the uptake of potassium protons. This leads to the acidification of extracellular spaces and activates the expansins which in turn reduce the stiffness of the cell wall. The reduction of stiffness in the cell wall allows for the potassium proton pump to intake as much water for cell expansion. ABP1 is marked as a cross with auxin being depicted as pentagons in Figure 3.5. Without ABP1, cells would not be able to interact with auxin at the plasma membrane.

3.5 Auxin and Environmental Stimuli

The naturally occurring plant hormone auxin is continuously moving throughout the biological system. It is transported in a polar method from shoot apex to root apex and is associated with plant growth and development. The next step in compiling a full comprehension of IAA is to examine factors that are outside the plant. An interesting feature of auxin transport is its responsiveness to directional light sources and the changes in the gravity vector [21]. Auxin reacts to its surroundings which is important in prolonging the longevity of the plant. The Cholodny-Went hypothesis states that the lateral transport of auxin across plant tissues under the influence of gravity or light is the main factor that promotes differential growth [21]. These

environmental stimuli alter the polar transit path of auxin, which induces the asymmetric growth associated with phototropism and gravitropism. The cellular and molecular interaction with auxin has been clarified. However, the translation of environmental stimuli to differential growth and the involvement of auxin have yet to be explained. An exhaustive approach will lay out the exact mechanisms behind the environmental responses of phototropism and gravitropism.

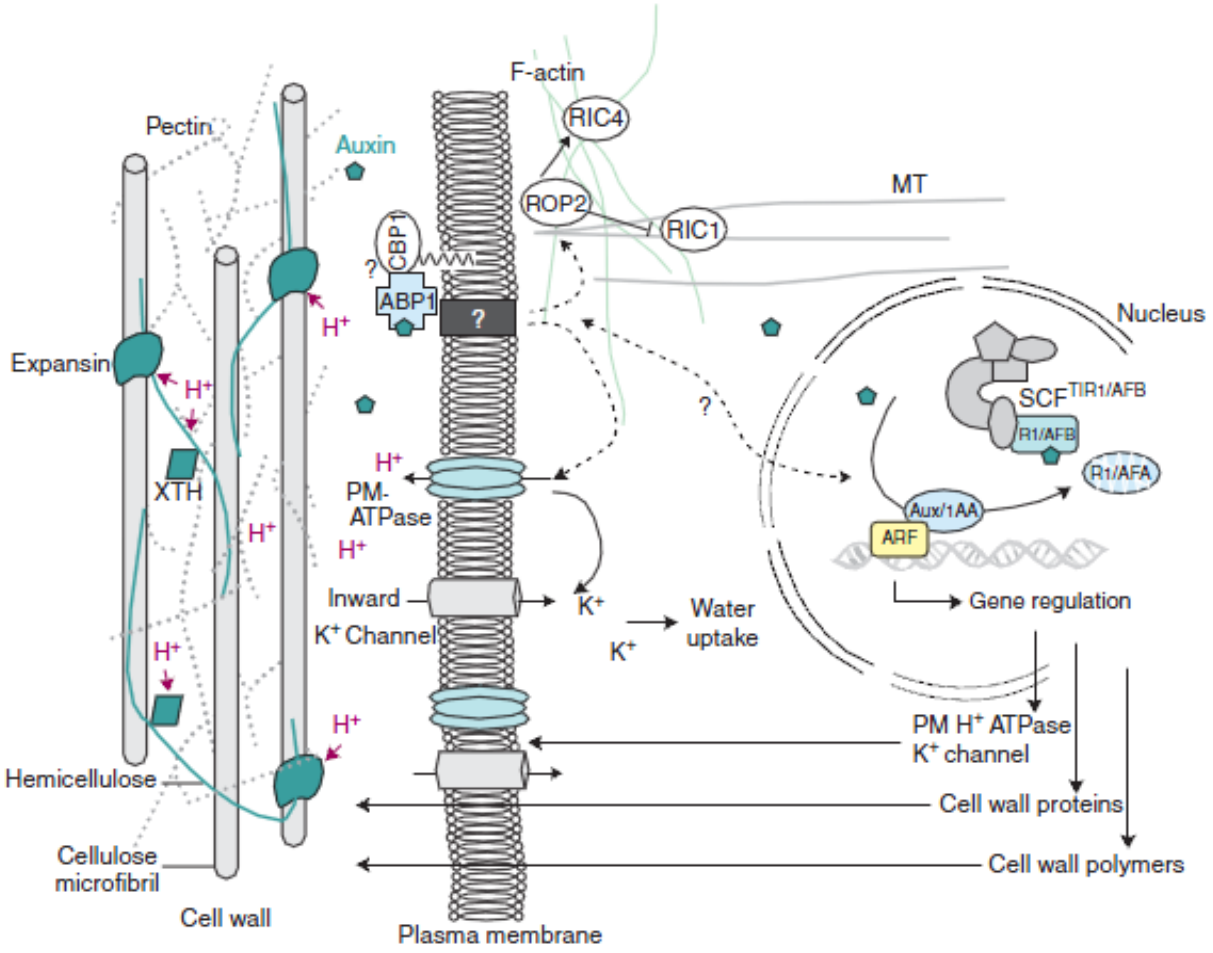

Figure 3.5 – The progression from the cell wall to the nucleus of a cell within the shoot is depicted. From left to right is the cell wall, followed by the plasma membrane and the nucleus of the cell. Auxin is perceived by ABP1 and activates the hydrogen proton pump. Hydrogen protons are extracted from the plasma membrane which induces a hyperpolarization and the activation of the potassium protons. Acidification results in the activation of the expansins and the cell wall stiffness is reduced. Water uptake takes place causing the cell to expand [22].

Chapter Four

The Underlying Mechanisms of Phototropism

4.1 Overview

Environmental stimuli can have varying effects on plants which can dictate different mechanisms throughout the biological system. An integral part of a plants environmental system is the consumption of light. Plants use light for the production of oxygen in the process of photosynthesis. Another process that is heavily dependent on the path of light and regulates plant motion is phototropism. As previously stated, phototropism uses light stimuli to mediate the movement of auxin from the light side to the shaded side of the plant [8]. However, before the cooperation with IAA, the perception of light within the plant must be examined. Plants contain different photoreceptive pigments that react to the spectra of light [31]. These light receptors register the direction and type of light that the plant is stimulated by. Once the photoreceptors have evaluated the perception of light, they induce a reaction within the cell. This reaction allows for the photoreceptors and certain signal transducers to communicate with one another [8]. Collaboration between the light receptors and signal transducers culminates in the movement of auxin throughout the plant. Lateral movement of auxin is initiated through a specific process and differential growth commences. The exact mechanics behind phototropism are further analyzed and discussed.

4.2 The Receptors of Light

4.2.1 Phototropins

The light spectrum consists of a multitude of lights that vary in color and have a distinct effect in certain environments. Plants are generally bathed in a full spectrum of light however, there are particular pigments within these biological structures that sense specific quantities of light [8]. In regards to phototropism, blue light is the most effective source of light that regulates such a process [8]. The photoreceptor that senses blue light specifically is called a phototropin. Through plant manipulation, phototropins have demonstrated a large range of physiological responses that include phototropism [9]. Phototropins are one of the main receptors of light that lead to the initialization of phototropism. They are the primary photoreceptors that are responsible the activation of phototropic responses [8]. Phototropins are a fundamental piece to unveiling the precise mechanisms behind phototropism.

Conceptually, the presence of a blue-light and its interaction with plant response has been around for almost two centuries. However, it was not until the 1990's that a pigment that absorbed blue light, now known as phototropin, was discovered [8, 9]. Experimentation performed on Arabidopsis thaliana plants distinguished two types of phototropins. PHOT1 and PHOT2 are autophosphorylating serine/threonine kinases with light-sensitive, oxygen-sensitive, or voltage-sensitive (LOV) domains [32]. The LOV domains bind flavin mononucleotide as chromophores for each of the phototropins [32]. PHOT1 and PHOT2 are essentially proteins that have the ability to absorb blue light wavelengths within the plant. Flavins absorb blue wavelength light strongly and are highly photoreactive [34]. Both phototropins are associated with the plasma membrane of the cell and are located in parenchyma cells found in the xylem and phloem [33].

4.2.2 Cryptochromes

Another photoreceptor that is associated with phototropins and plays a role in light absorption is the cryptochrome. Cryptochromes are blue light receptors that help to regulate phototropic response [8]. Similar to phototropins, cryptochromes consist of flavoproteins that are highly reactive to blue light [35]. But a key difference between the two photoreceptors is that cryptochromes are not necessary for the induction of phototropism [36]. They still compliment phototropins with the absorption and mediation for blue light responses. The mediation of blue light is a secondary function of cryptochromes while they are primarily involved in photomorphogenesis and the regulation of circadian rhythms [37]. CRY1 is the blue-light photoreceptor that was found during Arabidopsis mutation [37]. In these experiments there was a decrease in blue light signaling and was determined to be the absence of CRY1. Along with the management of blue light reception, cryptochromes have also been associated with the ability to prevent cell expansion. Stem irradiation with a pulse of blue light leads to the inhibition of hypocotyl growth [38]. Further testing revealed that CRY1 activates an anion channel at the plasma membrane causing a depolarization that prevents cell expansion [9]. Cryptochromes do not initiate phototropism but they are important in controlling blue light reception and the modulation of the phototropic pathway.

4.2.3 Phytochromes

A third photoreceptor has been identified in the regulation of light and how it is utilized by the plant system. Phytochromes are red light receptors that sense the wavelengths of both red and far-red light [39]. In plants, phytochromes are protein chemical compounds that consist of protein molecules that are identical [39]. The main distinction between phototropins,

cryptochromes and phytochromes is that the red light absorbed by the phytochromes does not actuate the process of phototropism. Arabidopsis experiments involving unidirectional red light aimed at the stem revealed that phototropism does not occur [9]. Despite not inducing phototropism, the use of red light and phytochromes has found another purpose. These red light receptors have been found to greatly increase phototropism that is induced by blue light [39]. Plant experimentation, including Arabidopsis species, used red-light pretreatments before testing for phototropic responses and results demonstrated a greater reaction to blue light induced curvature [40]. Similar to cryptochromes, phytochromes also mediate the inhibition of light-induced growth in hypocotyls [40]. Phytochromes consist of five PHY genes that have been identified through genetic manipulation of Arabidopsis plants [41]. The discovery of these PHY genes has also brought about the specific cellular location and activity of such photoreceptors. The PHY molecule has exhibited movement from the cytoplasm to the nucleus and interacts with the signaling pathway [41]. Essentially, PHY molecules alter the expression of specific genes responsible for plant adaptation dealing with growth and development due to the surrounding environment. Phytochromes work with cryptochromes in regulating stem growth and they also interact with phototropins to control and separate the intake of light [8]. Phytochromes and cryptochromes are two protein based pigments that assist phototropins with the perception of light and must be considered with the overall process of phototropism.

4.3 Cellular and Molecular Interaction

The development of phototropism begins with the perception of light and once registered a chain of events occurs starting at the molecular level. The main photoreceptive proteins PHOT1 and PHOT2 sense the light at the plasma membrane of the cell and localize in the

parenchyma cells within the stele of the plant stem [32]. CRY1 and PHY photoreceptors interact with PHOT1 and PHOT2 to discern different portions of the light spectra. After the light sensing pigments have been exposed to light the plasma membrane undergoes a distinct process. Phosphorylation is the process in which a phosphate group is added to a protein and has the ability to regulate protein function [31]. The stem registers the light through the photoreceptors and phosphorylation is induced bringing about a change in certain proteins. Genetic experiments involving the mutation of Arabidopsis plants have unveiled specific proteins that interact with phototropins. The non-phototropic hypocotyl3 (NPH3) and non-phototropic hypocotyl4 (NPH4) genes are proteins that interact with PHOT1 and PHOT2 [9]. These proteins are altered during the phosphorylation of the plasma membrane and as a result carry on the signal from the photoreceptors.

The relationship between PHOT1 and NPH3 has proven to be an important aspect to phototropism. PHOT1 is the main protein that senses light and uses NPH3 to establish a signal to continue throughout the plant. NPH3 binds to PHOT1 and is very early to act in defining the signaling pathway for blue light based phototropism [36]. Together, PHOT1 and NPH3 work to establish a signaling pathway in which other proteins are affected throughout the stem. After NPH3 binds with PHOT1, another reaction takes place at the plasma membrane. The auxin efflux carrier of the PIN family, PIN1, is found to localize at the base of cells within the cortex tissues [42]. Experiments demonstrated that after phototropic stimulation the PIN localization was interrupted more on the shaded side of the hypocotyl [42]. These results illustrate that the PIN1 proteins change localization to guide auxin to the shaded side of the plant where cell elongation occurs. PIN1 initiates the lateral auxin movement from the cortical layers out towards the tissues consisting of the epidermis. A regulator of the PIN1 gene is the auxin response factor

(ARF7) that is controlled by NPH4 proteins [43]. The cooperation between the NPH family and PIN family are crucial for phototropic response and the curvature that is associated with it.

(a) **(b)**

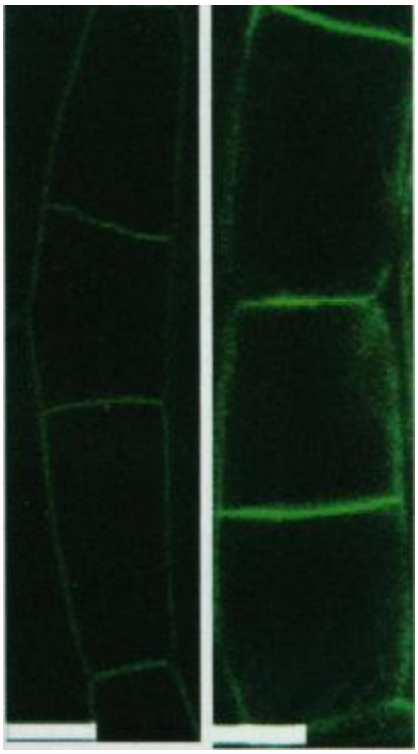

Figure 4.1 – The localization of the protein photoreceptor PHOT1 (green) as the plasma membrane of each cell is shown in the stem of an Arabidopsis plant. The epidermis layer in (a) depicts that PHOT1 is evenly distributed throughout the plasma membrane. PHOT1 is more localized at the apical and basal sections of the cells in the cortex layer in (b) [33]. White bar = 50μm.

4.4 The Signaling Pathway for Phototropism

There are many factors that contribute to the precise phototropic path in which auxin is regulated and distributed asymmetrically. Phototropism is initiated by the light receptor protein PHOT1 and PHOT2 registering the light gradient in the surrounding environment. CRY1 and PHY photoreceptors assist the phototropins in light mediation and proceed to regulate the light sensing signal. PHOT1 localizes to the plasma membrane of cells within the stem and initiates

the phosphorylation process previously mentioned. The addition of phosphate groups brings about the activation of the NPH3 proteins. These proteins encode other proteins similar in structure and localize at the plasma membrane to interact with PHOT1 [33]. NPH3 takes the signal originating from the phototropin pigments and uses it to transform the plasma membrane to further progress the signal. After the activation of the plasma membrane the PIN1 and PIN3 genes initiate lateral movement. These auxin efflux carriers move and begin to concentrate at the lateral walls of the plasma membrane establishing a gradient [23]. Once the gradient pathway has been formulated, auxin regulators are triggered to provide assistance for the lateral movement of auxin. Similar to NPH3, NPH4 is a protein that encodes ARF7 to continue the movement of the phototropic signal that began with the PHOT1 pigment [43]. Finally, the lateral transportation of IAA can follow the phototropic pathway that has been forged through each cellular layer. The activation and localization of explicit proteins at the plasma membrane of stem cells is crucial for phototropism.

4.5 The Role of Auxin in Phototropism

A global analysis of phototropism reveals that plant stems move in the direction of light with the shaded side of the stem undergoing deformation. The Cholodny-West hypothesis points at auxin and its lateral translocation to the shaded side of the plant as the driving force behind phototropism [9]. The signaling pathway that is actuated from the induction of light is the pivotal track in which IAA reacts and responds. From PHOT1 to ARF7, protein interactions at the molecular and cellular level make way for the plant hormone to laterally move along the stem system. The movement of auxin is not symmetric which creates the differential growth found with phototropic curvature. Both influx and efflux auxin carriers allow the hormone to enter and

leave the cell. As auxin moves throughout the cells, its effect on the plasma membrane and cell wall provide an environment for change.

Changes in the cell wall and turgor pressure within the cell are direct results of the interaction of IAA under the process of phototropism. At the plasma membrane, auxin activates an ATPase proton pump that induces the extraction of hydrogen protons [28]. Expansins within the cell wall decrease its stiffness and the potassium channels actuate bringing about the uptake of water [27]. Rigidity of the cell walls declines and the uptake of water leads to an increase in turgor pressure. Together, these processes result in the expansion of the cell. The summation of cell expansion on the shaded side allows for the plant stem to move in the direction of the light.

Figure 4.2 – The movement of auxin throughout the stem of an Arabidopsis thaliana plant is shown above in blue. In (a) the plant is illuminated from the right and the picture within depicts the concentration of auxin on the shaded side. Auxin efflux carriers are genetically altered in (b) revealing their importance in phototropism since there is no curvature and no movement of IAA to the shaded side of the plant [51]. Black bar = 50μm.

Chapter Five

Gravitropism: How Gravity Affects the Stem

5.1 Overview

Similar to light, gravity is another environmental factor that manipulates the movement and growth of plants. Gravitropism is the ability of plants to orient themselves in the direction of the gravity vector present in their surroundings [44]. A process of this nature is crucial to plant development and provides the biological structure a chance to fight the elements. Imagine a farmer with vast acres of crop and severe winds roll through the lands parting the crops in every direction but the vertical. If plants didn't react to the gravity sensor how would the farmer survive just one fight against Mother Nature? In the presence of gravity, the shoots of plants reorient themselves upwards while the roots curve downwards [45]. Gravitropism provides such biological structures with the opportunity to respond to gravity and straighten vertically. That previously mentioned farmer doesn't have to go out and fix the orientation of each plant.

The mechanisms behind gravitropic curvature give plants the power to react to various environmental stimuli that use the power of force. Hence understanding such a function and modeling it has the potential for further application. Parallel to phototropism, gravitropism is initiated by a specific part of the plant. Once the gravity vector is registered, interactions within the plant create a pathway for the gravitropic signal to propagate throughout the shoot of the plant. After the pathway is formed, the movement of auxin is activated and differential growth leads to curvature of the stem. The factors behind such curvature response will be discussed and laid out for future modeling.

5.2 The Perception of Gravity

On Earth, gravity is 9.81 meters per second squared or 1 g and is the constant force that must be considered in any domain. It is a vector that defines plant development and its cognizance is the rudimentary piece in the initiation of gravitropism. Research points to a distinct layer within the stem of plants that dictates the perception of gravity. In the innermost layer of the cortex a starch sheath that has been declared the endodermis is the definitive stratum that recognizes gravity [44, 45]. Within this tissue are specific organelles that move with the sensing of gravity. Amyloplasts, or statoliths, are dense organelles that contain starch granules and are located within each cell of the endodermis [46, 47]. The starch-statolith hypothesis combines the perception of gravity with the movement of amyloplasts. It states that amyloplasts sediment to the bottom of the endodermal sells and formulates a gravitropic signal [46]. In Figure 5.1, a vertical shoot from an Arabidopsis plant shows the sedimentation of amyloplasts in the endodermis.

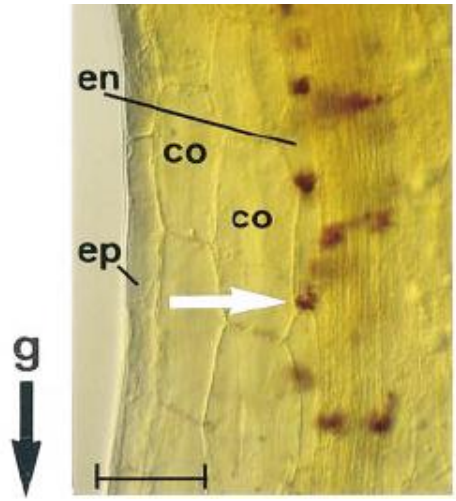

Figure 5.1 – An optical image of a vertically oriented shoot of an Arabidopsis thaliana plant is shown. From left to right the layers are the epidermis (ep), the cortex (co) and the endodermis (en). The black dots found within the endodermis are the amyloplasts that sediment during gravitropsim [44]. Bar = 50μm.

5.3 A Closer Look at the Cellular and Molecular Levels

The location in which the perception of gravity is measured has been instituted but the production of a signal and the path it travels has not. Again, the gradient of growth that is responsible for the curvature of the plant stem is a result from IAA interaction. There are specific genes at work at the molecular and cellular level that regulate the pathway for the transportation of auxin. Various genes a part of the Soluble NSF Attachment protein Receptors (SNARE) such as Shoot Gravitropic 2 (SGR2), SGR3, and SGR4/ZIG have been found in the early stages of gravity sensing [48]. These protein receptors interact with amyloplasts and their movement within the cell. Amyloplasts are surrounded by a vacuolar membrane within the vacuole of the cell and vesicle transportation to these vacuoles is mediated by SNARE [13, 48]. Essentially, the sedimentation of the amyloplasts will be recognized by these protein receptors and signal transference begins.

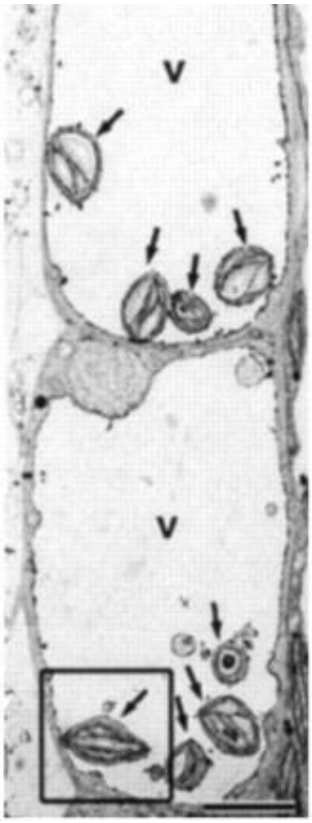

Figure 5.2 – Two cells within the endodermis of a vertically oriented Arabidopsis thaliana plant stem are presented. The lower case (v) illustrates the direction of the gravity vector which is downwards. The black arrows are pointing to the amyloplasts that have sediment to the bottom of each cell. Sedimentation of statoliths is essential to gravitropsim [48]. Black bar = 5µm.

Each cell possesses a cytoskeleton and research points to this structure as an important part of the gravitropic signal. This framework of proteins reinforces particular cell areas, influences cell shape and also mediates vesicle movement [13]. The cytoskeleton is located within the cytoplasm of the cell and consists of actin microtubules and microfilaments [13, 49]. It is at this location that amyloplasts collaborate with the actin network. The sedimentation of statoliths locally disrupts the dense actin network which is linked to the plasma membrane [49]. Disruption of the microtubules and microfilaments results in a modification of the tension. This change in tension of the actin network induces mechanosensitive ion channels at the plasma membrane of the cell [26]. The change in tension of the cytoskeleton due to the amyloplasts activates the plasma membrane via ion channels that allow the cell to sense the gravity vector. The signal is transferred from the sedimentation of the statoliths to the entire cell.

5.4 Auxin Regulation in Gravitropism

After the endodermis has detected the gravity vector with amyloplast sedimentation interacting with the cytoskeleton, a mediated channel is formed allowing for a lateral gradient of auxin. Similar to phototropism, gravitropic curvature is induced by the signaling pathway that translates the movement of IAA from the vascular tissues to the epidermal layer of the plant stem. Key elements that participate in the guidance of IAA are the auxin efflux carriers that interact with the cell. Such carries provide auxin with the distinct ability to move laterally throughout the plant. The movement is not symmetrical resulting in differential growth which is the driving factor behind curvature. What is important to note is that the distinct proteins used for auxin movement vary from plant to plant.

5.4.1 Movement of Auxin in Arabidopsis thaliana Plants

A species of plant that has contributed significantly to the examination of gravitropism is the Arabidopsis thaliana plant. Numerous experiments, many involving genetic manipulation, have provided insight on how IAA is laterally transported. An auxin gradient is attained by the redistribution of auxin efflux carriers that are a part of the PIN-FORMED (PIN) family and ATP binding cassette (ABC) transporters [23]. The protein genes PIN1, PIN3, PGP1, and PGP19 are the important efflux carriers that interact with auxin [9, 23, 50]. This redistribution of IAA transporters enables polar auxin movement across the elongation zone to the layers of the cortex and epidermis which establishes the asymmetric growth associated with curvature [51].

Polar localization of PIN proteins is a process that is regulated by the dynamic cycling of such proteins between endosomes within the cell and its plasma membrane [13]. PIN1 is a protein that localizes to the plasma membrane asymmetrically and helps to mediate the gravitropic signal pathway. Experiments that inhibited vesicle movement revealed the power behind PIN1 and how auxin transport was reduced [13]. The role of PIN1 for the movement of IAA is important to the process of differential growth found in gravitropism. PIN3 is another protein efflux carrier that is found within the endodermis of the plant shoot [51]. This gene also has the responsibility of promoting the lateral movement of auxin from the endodermis to the epidermis [50]. PIN3 is localized to the inner longitudinal wall and base of the plasma membrane within the endodermis [51]. Since these PIN genes have a dynamic nature, they will reorient themselves according to the gravity vector. The endodermis is a crucial layer to the plant since it both contains amyloplasts and auxin efflux carriers that move and are necessary for lateral translocation.

Lastly, the lateral transit of auxin occurs and drives the gravitropic curvature in the direction of the gravity vector. IAA moves from the apex of the shoot to the apex of the root in a polar fashion and now laterally from the stele to the epidermis under gravity stimulation. Once auxin has reached the proper layers, cell expansion takes place and curvature due to the lateral, asymmetric movement of auxin is initiated. The proton pump channels at the plasma membrane are induced and the structure of the cell begins to change. Stiffness of the cell walls in which auxin reacts with is reduced and the turgor pressure within the cell is increased due to the uptake of water. The combination of the two processes allows for cell expansion, which leads to overall global curvature.

5.4.2 Lateral Auxin Movement in Soybeans

Another plant that has undergone testing and examination under the conditions of gravitropism is the soybean. In the shoots of soybean plants, gravity stimulation is followed by rapid asymmetry in the accumulation of a group of auxin-stimulated mRNAs called small auxin up-regulated RNAs or SAURs for brevity [52]. SAURs interact with auxin to regulate its movement and provide direction within the plant cells. In vertical soybean seedlings, the SAUR gene expression is symmetrically distributed and curvature due to differential growth is nonexistent [52]. However, a horizontal orientation changes things and a difference can be seen. After twenty minutes of the soybean being place in the horizontal direction, SAURs begin to accumulate on the lower half of the hypocotyl and twenty five minutes later gravitropic bending is visibly evident [52]. The movement of the SAURs to the lower flank of the shoot assists in the lateral movement of auxin to the outermost layer. IAA follows the signal path mediated by

SAURs and proceeds to interact with the structure of the cell. Reduced cell wall stiffness and the increase in turgor pressure produces cell expansion and curvature at the global level.

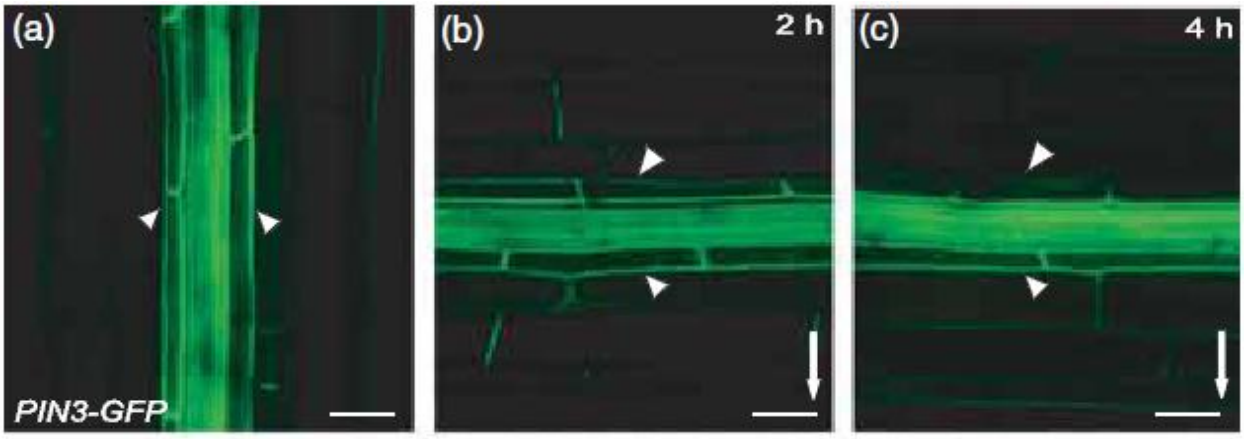

Figure 5.3 – Examination of the endodermis of an Arabidopsis thaliana plant stem shows that PIN3 localizes to the plasma membrane of the cell. First, the stem in (a) is vertically positioned and PIN3 is found at the plasma membrane of the endodermis (white arrowheads). The stem is horizontally oriented in (b) and (c) with the gravity vector marked by the white arrow. From 2 hours (b) to 4 hours (c), the concentration of PIN3 has moved from the top half of the stem to the lower half thus establishing lateral movement of PIN3 within each layer [67]. White bar = 50μm.

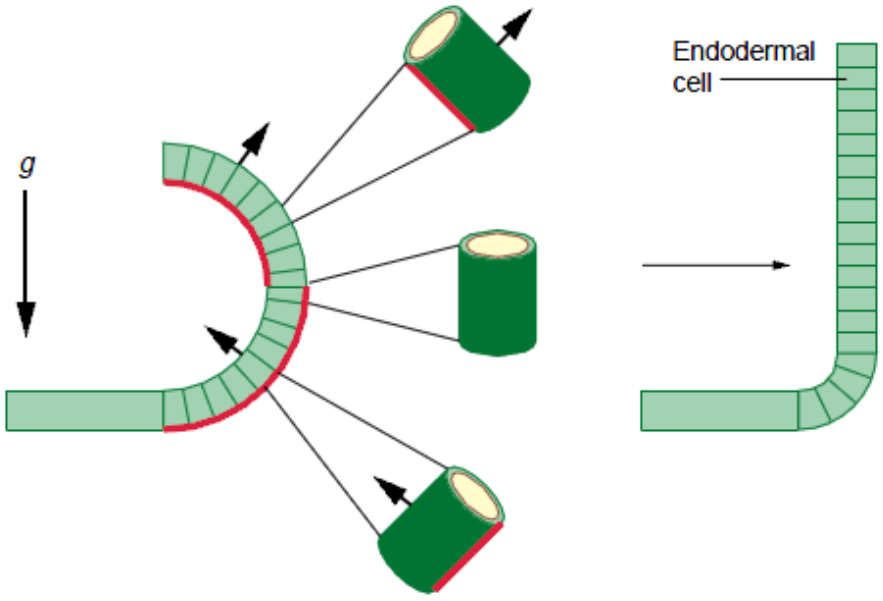

Figure 5.4 – A global view of endodermal cells within a plant and how certain areas of the stem respond to gravitropism is depicted. The horizontally oriented cells will lead to upward bending from gravity stimulation. The red indicates the sides of the layer that will undergo cell expansion and further promote upward curvature [45].

Chapter Six

A Model for Tropisms

6.1 Introduction

Before the successful implementation of a model can be instituted, the comprehension of the mechanisms behind the specific function under examination must be precise. An in-depth analysis of phototropism and gravitropism has provided the necessary tools to embark on the formulation of a proper model. From the global to molecular level, the plant hormone auxin has been the decisive element controlling the curvature that is induced via each tropism. In phototropism, light receptors induce the lateral movement of auxin to the shaded side of the hypocotyl thus actuating curvature. While gravitropism uses the force of gravity, which is perceived by the endodermis, to localize auxin at the lower region of the stem that is closest to the ground with curvature ensuing. Research points to the uptake of water and the reduction of cell wall stiffness through the interaction with auxin as the leading factors attributed with cell expansion. Reduced cell wall stiffness and internal turgor pressure allow the cell to expand in response to environmental stimuli.

As each cell expands in the area of the asymmetric auxin distribution, together they make up the total curvature that the plant experiences. The first step in creating a model that incorporates the gradient of auxin is to examine a single cell and mechanically define the physics of its expansion. This initial approach will be defined as the micro kinematics while macro kinematics will involve the overall expansion and curvature affiliated with each tropism. From that point, relationships between auxin, cell wall stiffness, and turgor pressure will be derived

and installed into the macro-micro equations. Further examination of the auxin gradient will involve the separation between phototropism and gravitropism. The combination of each relationship will result in a model that distinctly defines curvature with respect to the movement of auxin during the processes of phototropism and gravitropism.

6.2 Physics Behind Single Cell Expansion

The first step in amassing a comprehensive model for cell expansion is to examine the motions of a single cell. Figure 6.1 depicts a shoot of a plant with a diagonal orientation which a small portion is taken from and zoomed in. The initial shoot configuration, S_0, illustrates the coordinate system that will be implemented into the model. The angle φ is the measured angle between the vertical axis and the stem orientation. This angle is taken to be positive going from the vertical to the right and will be crucial for gravitropsim. The centroidal axis *CA* of the shoot is marked by the red dashed line and the initial length of a cell is given the label of l_0 in Figure 6.1.

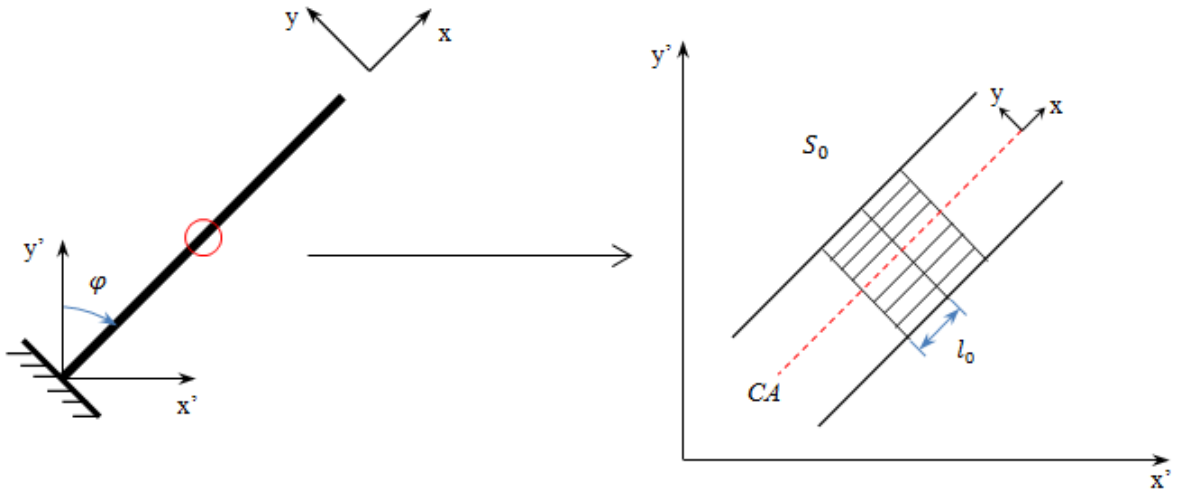

Figure 6.1 – Global view of a diagonally oriented stem with a zoom-in of the cellular level of the stem.

Strain, ε, is a measure of deformation that is defined by the change in length over the original length and is a dimensionless unit of measure. To measure the strain of the expansion of a cell two properties of deformation must be considered. Both axial and bending deformations are required for a total evaluation of strain of the cell. Axial deformation is shown in Figure 6.2 as the stem is elongated in the x direction.

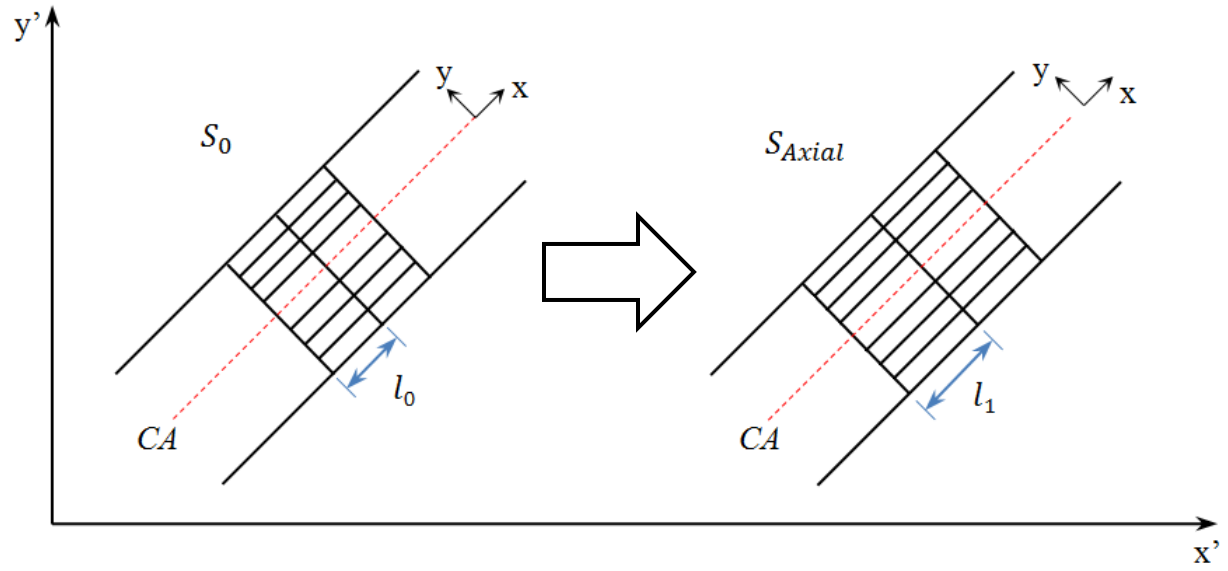

Figure 6.2 – The stem is undergoing axial deformation from S_0 to S_{Axial}.

With reference to figure 6.2, the equation for axial strain, ε_A, is defined as

$$\varepsilon_{Axial}(x) = \frac{l_1(x) - l_0(x)}{l_0(x)} \tag{6.1}$$

Once axial strain has been accounted for, the strain that occurs due to bending can be examined. Each cell in the lateral direction will have the same amount of axial strain before the effects of bending are induced. Upon curvature, the cells along the centroidal axis *CA* of the stem will remain the same in length. However, cells above and below the center axis will either elongate or shrink which is dependent on the direction of curvature. In Figure 6.3, the stem is undergoing bending deformation after the effects of axial deformation have been actuated.

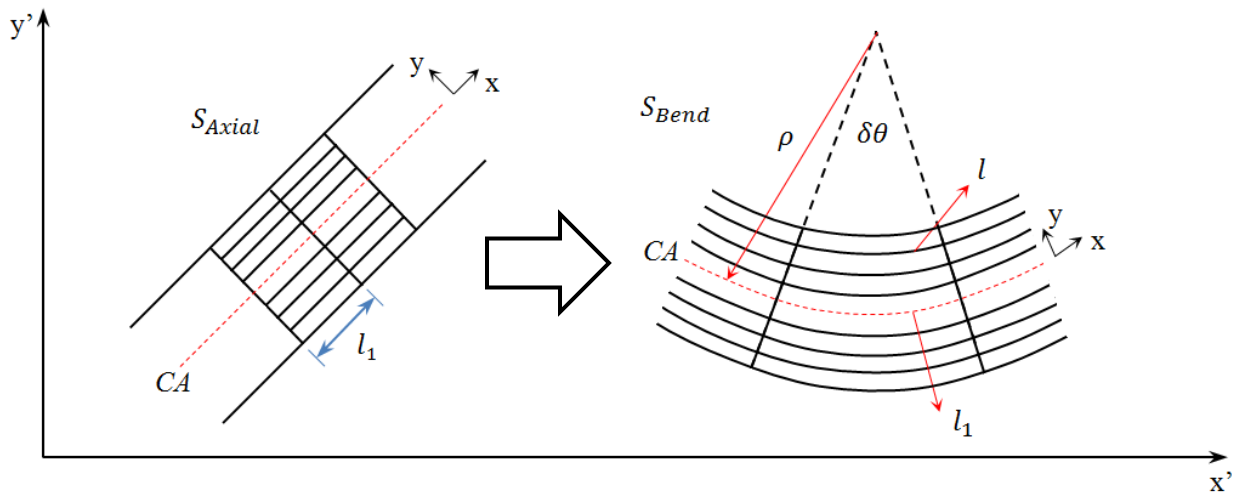

Figure 6.3 – Deformation due to bending (S_{Bend}) is followed after axial deformation (S_{Axial}) has occurred. The length of the cells at the centroidal axis *CA* of the bending stem is equal to the length of the cell after axial deformation.

Figure 6.3 reflects the deformation due to axial elongation (S_A) and bending (S_B), which are both a necessity when defining total deformation of the cells within the stem. In the bending deformation configuration S_B, ρ is the radius of curvature and $\delta\theta$ is the angle of rotation being examined. Again, the centroidal axis is marked by the red dotted line located at the center of the plant shoot. The strain due to bending involves the roles of l and l_1 in terms of the radius of curvature ρ and the differential angle $\delta\theta$. In relation to Figure 6.3

$$l_1 = \rho\delta\theta \tag{6.2}$$

$$l = (\rho - y)\delta\theta \tag{6.3}$$

The strain due to bending is equal to

$$\varepsilon_{Bend} = \frac{l - l_1}{l_1} \tag{6.4}$$

Using equation 6.2 and 6.3 the equation of bending strain turns into

$$\varepsilon_{Bend}(y) = \frac{(\rho - y)\delta\theta - \rho\delta\theta}{\rho\delta\theta} = -\frac{y}{\rho} \tag{6.5}$$

Then, bringing the variable of κ for curvature

$$\kappa = \frac{1}{\rho} \tag{6.6}$$

and subbing in equation 6.6 into 6.5, the strain equation turns into

$$\varepsilon_{Bend}(y) = -\kappa y \tag{6.7}$$

The total strain due to both axial and bending deformations is shown as

$$\varepsilon_{TOT} = \varepsilon_{Axial} + \varepsilon_{Bend} \tag{6.8}$$

With the substitution of equations 6.1 and 6.7, the total strain equals

$$\varepsilon_{TOT}(x, y) = \frac{l_1(x) - l_0(x)}{l_0(x)} - \kappa(y)y = \varepsilon_0(x) - \kappa(y)y \tag{6.9}$$

since ε_{Axial} is with respect to the axial plane of the plant stem which is in the x direction and ε_{Bend} is in the y direction. The total strain equation in 6.9 is an integral part of the model and will be further modified.

6.3 Turgor Pressure and Compliance of the Cell

Two important factors that are a necessity in the process of cell expansion are the internal turgor pressure and the weakening of the cell wall. Turgor pressure increases in the cell due to an uptake of water while the cell wall loosens due to a decrease in stiffness. Each of these processes is affected by the asymmetric distribution of auxin. Essentially, turgor pressure and cell wall compliance are functions of auxin. The lateral movement of auxin with respect to the shoot coordinates puts it in respect to the y axis. Before any stimulation due to environmental factors, auxin is being transported in a polar direction, which means that it is initially moving with respect to the x axis of the shoot. The same is going to occur with the both turgor pressure and compliance of the cell. First, the derivation of the pressure equation will be introduced.

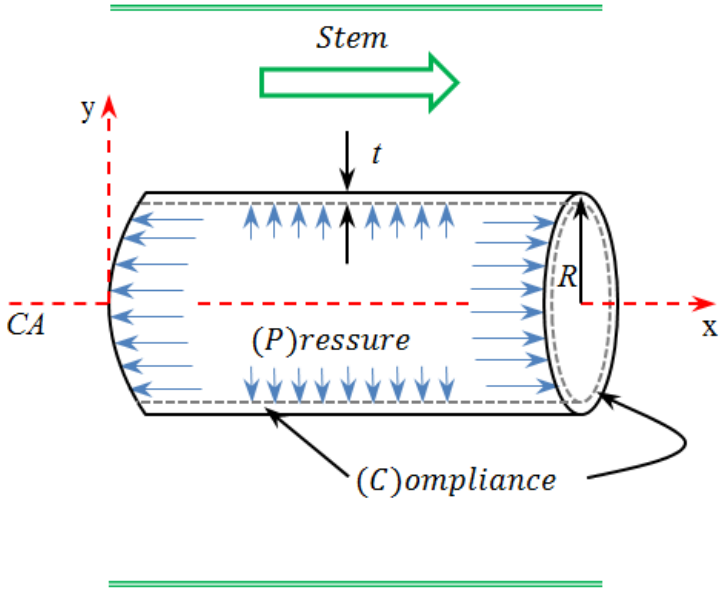

Figure 6.4 – An individual cell from a horizontally oriented stem with the internal turgor pressure P marked by the blue arrows and the compliance C shown at the cell wall. The radius R and thickness of the cell wall t are constants important to the model.

The equation of turgor pressure will be established with the examination of the single cell above. In Figure 6.4, the internal turgor pressure P is indicated by the blue arrows and it is located at the cell wall. Before any response to stimuli takes place, there is an initial turgor pressure P_0(x) within the cell. A vertically oriented shoot shows symmetric growth and has no curvature indicating the need for an initial pressure. In terms of this model, the greatest force from pressure will be found at the cell walls normal to the x-axis. This is the case because the walls parallel to the x-axis reduce in stiffness and increase in compliance allowing for overall cell expansion. The symmetric pressure gradient changes as auxin laterally moves and interacts with the cell. $P_{,Y}$(x) is the pressure variable that is dependent upon the concentration of auxin as it responds to environmental stimuli. Together, the following equation represents the pressure gradient with respect to the y axis

$$P(x,y) = P_0(x) + P_{,Y}(x) * y \tag{6.10}$$

To establish the concept of compliance in one dimension, a simple manipulation of Hooke's Law is used, in which a relationship between stress and strain has been defined. Strain in terms of compliance and stress is equal to

$$\varepsilon = C\sigma \tag{6.11}$$

where C is the compliance, usually defined as the inverse of the stiffness ($C = \frac{1}{E}$). This simple manipulation of Hooke's Law allows for simpler implementation of this strain-stress relationship as the model comes together. Compliance takes into account the maneuverability of the cell walls surrounding each cell. The cell walls that are affected the most by auxin are parallel to the centroidal axis and allow for expansion. A higher compliance means that the cell walls can expand more easily since there is a reduced stiffness. There is an initial compliance that is related to the internal turgor pressure. When the plant is not under the effect of any stimuli and vertically oriented there is an initial compliance C_0 with respect to the x-axis. As auxin moves laterally through the plant stem, it activates expansins within the cell wall allowing compliance $C_{,Y}$ to increase in relation to the auxin concentration. $C_{,Y}$ is directly related to the concentration of auxin with respect to the y-axis. The following equation represents the overall compliance of the cell wall

$$C(x, y) = C_0(x) + C_{,Y}(x) * y \tag{6.12}$$

Two equations representing internal turgor pressure and compliance of the cell walls have been formulated. The next step is to relate these two functions to the strain-stress equation found in 6.11. In terms of stresses acting on the cell, the internal turgor pressure used for cell expansion is the only force considered. This relationship is clarified as

$$\sigma = \lambda P \tag{6.13}$$

thus turning equation 6.11 into

$$\varepsilon(x, y) = C(x, y) * (\lambda P(x, y)) \tag{6.14}$$

With equation 6.14, the equation for turgor pressure 6.10, and that of compliance, 6.12 can be subbed in

$$\varepsilon(x, y) = \lambda(C_0(x) + C_{,Y} * y)(P_0(x) + P_{,Y} * y)$$

$$= \lambda C_0 P_0(x) + \lambda\big(C_0(x)P_{,Y} + C_{,Y}\, P_0(x)\big) * y + \lambda(C_{,Y}\, P_{,Y}) * y^2 + \mathrm{O}y^3 \tag{6.15}$$

The value of y is in the range of micrometers (μm) which is very small. Thus the second term will approach an infinitely small number with the increase of higher order terms upon further analysis. Therefore, the higher order terms $\lambda(C_{,Y}\, P_{,Y})y^2$ and $\mathrm{O}y^3$ will be omitted so that equation 6.15 becomes

$$\varepsilon(x, y) = \lambda C_0 * P_0(x) + \lambda\big(C_0(x) * P_{,Y} + C_{,Y} * P_0(x)\big) * y \tag{6.16}$$

Through various equation manipulations, the strain equation 6.16 is now represented through values involving compliance and turgor pressure of the plant cell. C_0, P_0, and λ are constants that represent the initial cell compliance, initial turgor pressure within the cell and the relationship with stress and pressure, respectively. $P_{,Y}$ and $C_{,Y}$are functions of auxin that rely on its movement throughout the plant. Each of these values help to simplify the strain equation and provides a clear direction for the model.

6.4 Simplification of Macro Equations

The strain equations for a single cell with the inclusion of turgor pressure and cell wall compliance have been determined. By comparing the two sets of strain equations 6.9 and 6.16, relationships are determined and further modeling assistance is established. The following equations for axial strain and curvature can be respectively deduced

$$\varepsilon_0(x) = \lambda C_0(x) * P_0(x) \tag{6.17}$$

$$\kappa(x, y) = -\lambda\big(C_0(x) * P_{,Y} + C_{,Y} * P_0(x)\big) \tag{6.18}$$

From known effective derivations, further associations between curvature κ, initial strain ε_0, and distinct derivatives of deformation establish a link that can be manipulated and simplified.

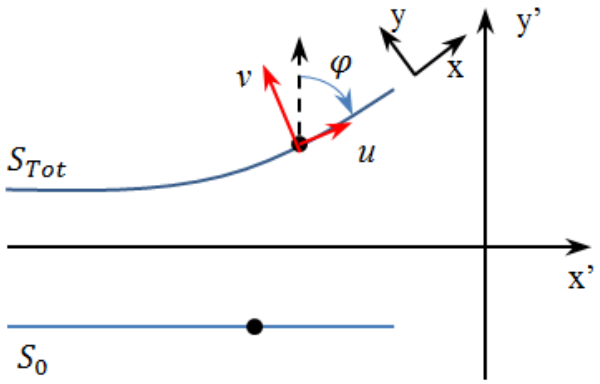

Figure 6.5 – A breakdown of an axial stem before S_0 and after bending S_{Tot}. The term v is for deflection along the y-axis and u is for strain along the x-axis with respect to the curve of the stem. φ is the angle from the vertical as well as the slope of the stem as it curves.

As Figure 6.5 for reference, the following relationships are formed

$$\kappa = \frac{d\varphi}{dx} \tag{6.19}$$

$$\varepsilon = \frac{du}{dx} \tag{6.20}$$

where curvature and strain are put in terms of *u* and *v*. The inclusion of large deflections brings about another approach to determine the main functions that will be associated with phototropism and gravitropism. Equations 6.19 and 6.20 allow for comparisons to be drawn hence the next set of relationships can be formed.

$$\frac{d\varphi}{dx} = \kappa(x,y) = -\lambda\big(C_0(x) * P_{,Y} + C_{,Y} * P_0(x)\big) \tag{6.21}$$

$$\frac{du}{dx} = \varepsilon_0(x) = \lambda C_0(x) * P_0(x) \tag{6.22}$$

Through integration of the slope φ and the use of another relationship with $\frac{dv}{dx}$, plant stem curvature can be produced. With a final set of integration, the function for deflection v can be determined and used for either plant tropism. The progression of these equations will lead to differential equations that may need the utilization of computer power. Numerical integration is a powerful tool and allows for a more effective method to solving such differential equations as opposed to standard integration. In the equations above, C_0 and P_0 are constants that involve the initial values for compliance and turgor pressure. The only values that are affected by auxin movement are $P_{,Y}$ and $C_{,Y}$. The unification of the two strain equations has allowed for a clear cut model that just needs the interaction of auxin with respect to each tropism.

6.5 Turgor Pressure and Compliance as Functions of Auxin

6.5.1 Auxin in a Mathematical Sense

Plant motion is largely dependent on the movement of auxin, both in the polar and lateral directions, which makes it a very crucial part of the model. As previously stated, IAA is a naturally occurring plant hormone that moves from the shoot apex towards the root apex. Before

the presence of environmental stimuli, auxin is flowing throughout the plant. Therefore, there is an initial concentration of auxin that is found within the plant and must be introduced to the model. Since the initial concentration of IAA is moving in a polar direction, it is moving with respect to the x-axis in this model. Plant curvature due to environmental stimuli response is due to the asymmetric lateral movement of auxin. Therefore, the lateral motion of auxin is in the y-axis direction and is dependent on the stimuli that the plant is reacting to. In the following equation, a_0 and $a_{,Y}$ are the initial concentration of auxin and the concentration of auxin with respect to the specific tropism

$$a(x, y) = a_0(x) + a_{,Y}(x) * y + High\ Order\ Terms \tag{6.23}$$

Equation 6.23 is the total concentration of auxin as it moves through the stem of the plant. Ignoring the high order terms, Equation 6.23 becomes a first order approximation that allows the assumptions for such equations to be linear. With higher order terms, the margin for error increases and can harm the overall functionality of an important equation. From this equation, comparisons can be made with compliance and turgor pressure which are the essential pieces for cell expansion.

6.5.2 Turgor Pressure as a Function of Auxin

Uptake of water is a vital function for plant cells and as a result this leads to the importance of turgor pressure. Plant physiology defines turgor pressure as the force exerted on the cell wall via the water within the cell membrane. It is an important force that helps to maintain cell structure as well as provide assistance in cell expansion. Turgor pressure and compliance are the two essential factors that contribute to cell expansion. Similar to compliance, turgor pressure is also related to the movement of the concentration of auxin. Before a response

to stimuli occurs, auxin is already moving in a polar direction and each cell contains an initial amount of turgor pressure P_0. Auxin also moves with response to stimuli and with a differential gradient ATPase proton pumps are activated at the plasma membrane of the cell. This activation leads to the uptake of water and the increase of turgor pressure $P_{,Y}$. The overall function of turgor pressure with respect to auxin is stated as Equation 6.24, where

$$P = f(a(x,y)) = \bar{P} + \beta a \tag{6.24}$$

In this first order approximation, $\bar{P}$ is the initial turgor pressure at the cell wall, β is the linear relationship between turgor pressure and the concentration of auxin, and a is the function of auxin with respect to plant tropisms. With the substitution of equation 6.23 into 6.24, the turgor pressure derivation turns to

$$P(a(x,y)) = \bar{P}(x) + \beta a_0(x) + \beta a_{,Y} * y \tag{6.25}$$

Comparison to equation 6.10 yields

$$P_0(a,x) = \bar{P}(x) + \beta a_0(x) \tag{6.26}$$

$$P_{,Y}(a,y) = \beta a_{,Y} \tag{6.27}$$

Again, it can be clearly seen that there is only one variable that has yet to be determined and drives the change in turgor pressure. $a_{,Y}$ is the quantity that describes the lateral movement of auxin under the specific tropism that induces curvature. The other variables are constants that are found either at initial conditions ($\bar{P}(x)$, $a_0(x)$) or relationships dependent on auxin movement (β). In Figure 6.6, the linear assumption of turgor pressure in relation to the increase of auxin is shown. The function behind β may be more complex than a linear correlation but is assumed to be linear for modeling simplicity. Like turgor pressure, compliance follows a similar path.

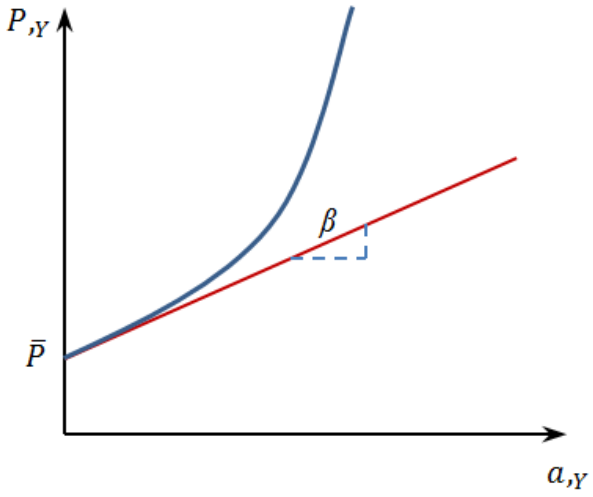

Figure 6.6 – The assumed linear relationship between auxin and turgor pressure is shown.

6.5.3 Compliance as a Function of Auxin

Research has revealed the role of auxin and the effects it has on the cell walls of cells within the plant stem. Auxin interacts with the cell wall by activating expansins that reduce the stiffness of the cell wall and increase its compliance. Thus, cell wall compliance is a function of auxin concentration and relies on the activation of those cell wall loosening proteins. A first order approximation allows a linear relationship to be formed between compliance and auxin which is

$$C = f(a) = \bar{C} + \alpha a \tag{6.28}$$

$\bar{C}$ is the compliance of the cell before auxin has interacted with the cell walls. α is the linear relationship between the concentration of auxin and the compliance of the cell walls. Equation 6.24 is then substituted into 6.28 resulting in

$$C(a(x,y)) = \bar{C}(x) + \alpha a_0(x) + \alpha a_{,Y} * y \tag{6.29}$$

Now the equations of 6.15 and 6.28 can be compared, deriving the following equations

$$C_0(a,x) = \bar{C}(x) + \alpha a_0(x) \tag{6.30}$$

$$C_{,Y}(a, y) = \alpha a_{,Y} \tag{6.31}$$

The only value that is not known is the concentration of auxin $a_{,Y}$ as it moves with response to specific environmental stimuli. $\bar{C}(x)$, α, and $a_0(x)$ are constant values that depend on the initial conditions of the cells within the plant stem. The alpha constant is dependent upon the linear correlation between compliance and auxin shown in Figure 6.7. This linear assumption limits the model but decreases complexity initially since the relationship could be more robust. These equations can now be implemented into the macro equations previously formulated rendering simpler equations that will be easier to manipulate. The only step left is to derive clear equations for laterally moving auxin with respect to the plants surroundings.

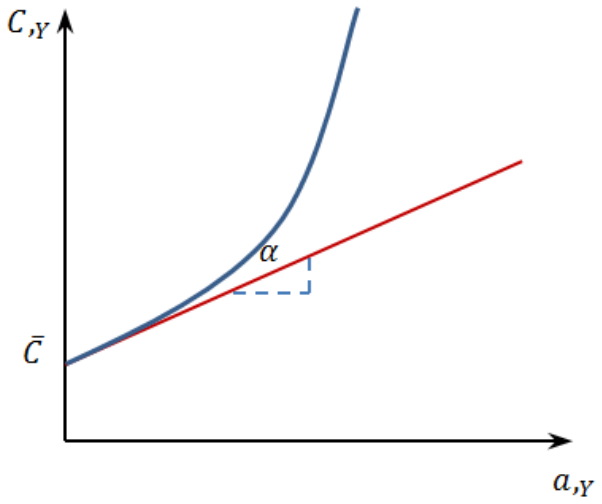

Figure 6.7 – The relationship between compliance and auxin is shown. A linear assumption is made for model simplification.

6.6 Defining Auxin in Phototropic and Gravitropic Terms

6.6.1 Overview

The final piece to the puzzle is to define functions that best exemplify the lateral motion of auxin under the influence of environmental stimuli. The Cholodny-Went hypothesis states that

it is the lateral transportation of auxin from the stele to the epidermis that creates the differential growth gradient that induces curvature [9]. This lateral movement is found in both phototropism and gravitropism. Stated earlier, phototropism is the ability of the plant to register light and to readjust itself in the direction of light. The cells in the stem expand on the shaded side, where auxin has been laterally transported to, allowing for a curvature response in the direction of light. Gravitropism occurs when the plant senses the gravity vector actuating curvature against the direction of gravity. The cells in the stem expand on the side that is closest to the ground since that is where auxin localizes. Equations for each tropism are important to this model and are formulated from experimental information.

6.6.2 Phototropism and Auxin

Light is the main stimulus behind the mechanisms of phototropism and is vital to plant life. Upon the recognition of light, auxin is laterally transported from the lit side to the shaded, less illuminated region of the stem. The intensity and location of the light source that is affecting the plant stem are crucial elements for modeling and must be properly examined. Figure 6.6 illustrates the basic plant movements behind phototropism and how they affect the concentration of auxin.

Research and experiments involving genetic mutation have demonstrated the importance of auxin movement with respect to light. Arabidopsis thaliana plants have been used for phototropism testing and it was found that light above the stem or the absence of light produces a symmetric distribution of auxin [41]. For modeling purposes, the equation that involves auxin with respect to phototropism must satisfy the condition that equal light in each direction or no light at all will result in zero curvature since IAA is symmetrically distributed. It is when the

light source moves to a certain side or increases on a certain side that the lateral movement of auxin is initiated.

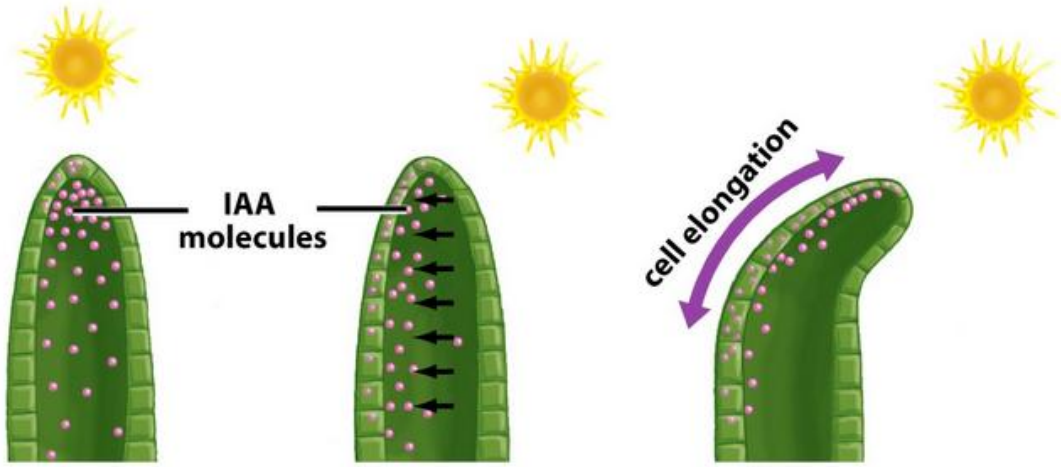

Figure 6.8 – The direction of light is a critical aspect of phototropism and guides the lateral movement of IAA from the light side to the shaded side. On the left, the plant stem is vertically oriented with the light directly above the stem showing an even auxin distribution. Light has moved to the right and auxin is shown moving to the left in the middle plant stem. Such movement induces cell elongation on the shaded side with expansion in the direction of the light as depicted in the last stem on the right [68].

Intensity is the measure of light and will be a part of the equation that relates phototropism to the lateral movement of auxin. It is measured as the concentration of light (P) divided by the area (A) upon which it is illuminating. The following equation breaks down the formulation of intensity

$$I = \frac{P}{A} \tag{6.32}$$

In Figure 6.7, a plant stem is shown vertically and then is reoriented after the intensity of I_2 is greater than that of I_1. What is important to note is that as the shoot is moving in the direction of light, the area in which it shines on increases. This increase in area is dependent upon the angle φ and thus creates another geometric relationship to analyze. Equation 6.33 alongside Figure 6.7 depicts the area relationship as follows

$$A = \frac{A_0}{\cos(\varphi)} \tag{6.33}$$

As the plant bends towards the light, the area with respect to the stem increases and is shown with the above equation. The combination of Equations 6.32 and 6.33 reveals an important piece to the model.

$$I = \frac{P}{A_0}\cos(\varphi) \tag{6.34}$$

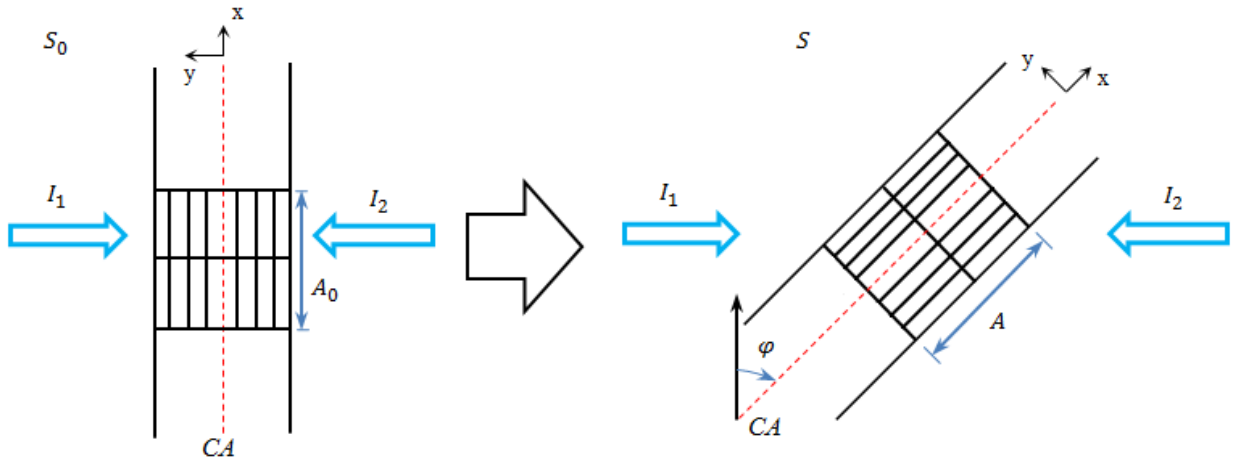

Figure 6.9 – A vertically oriented plant stem S_0 which depicts the light intensity as I_1 and I_2 that is used for the model. With I_2 being greater than I_1, the shoot S will reorient itself in the direction of I_2.

Together with Figure 6.9, Equation 6.34, and the information known about phototropism, the following equation represents auxin movement as a function of light intensity

$$a_{,Y}\,(x,y) = \xi\big(I_2(x) - I_1(x)\big) * \cos(\varphi) \tag{6.35}$$

This equation satisfies the experimental knowledge known about phototropism and auxin. If $I_2 = I_1$, indicating the light intensity is the same on either side then the concentration of auxin is symmetric and $a_{,Y} = 0$. If there is a difference between the two light sources, a gradient is

formed and the concentration of auxin is affected. When $a_{,Y} > 0$, that signifies that the light intensity on the right I_2 is greater than on the left I_1 and curvature will bend the plant stem to the right and vice versa. Light intensity I is measured as $\frac{\mu mol}{m^2 s^1}$ and can also be measured as lux $\left(\frac{lm}{m^2}\right)$, which is lumen per square meter [61]. Also, when the shoot reaches a horizontal position the angle φ will be $\pm\frac{\pi}{2}$ rendering $a_{,Y} = 0$ which is true because the plant is in the same direction as the light source. It will be the same condition that is shown in the first stem of Figure 6.6. ξ is the light-mediated driving force for the lateral motion of auxin which is measured in $\frac{\mu mol}{m \cdot lm}$. It is the area in which auxin moves away from the illuminated side of the plant towards the shaded side. This is an important element in the process behind phototropism.

6.6.3 Gravitropism and Auxin

Recognition of the gravity vector is the key component to the process of gravitropism and how it affects plant curvature. Gravity on Earth is always oriented downward and it is the responsibility of the plant to reorient itself with respect to gravity. Vital data points to the endodermis, which contains starch-filled particles called amyloplasts that sediment to the respective bottoms of each cell [46]. The sedimentation of these amyloplasts leads to a change within the cell and induces signal transduction. The molecules interact with each other and auxin begins to laterally move across the cell layers of the plant shoot. As previously stated, this lateral movement of auxin induces curvature via the increase of turgor pressure and cell wall compliance.

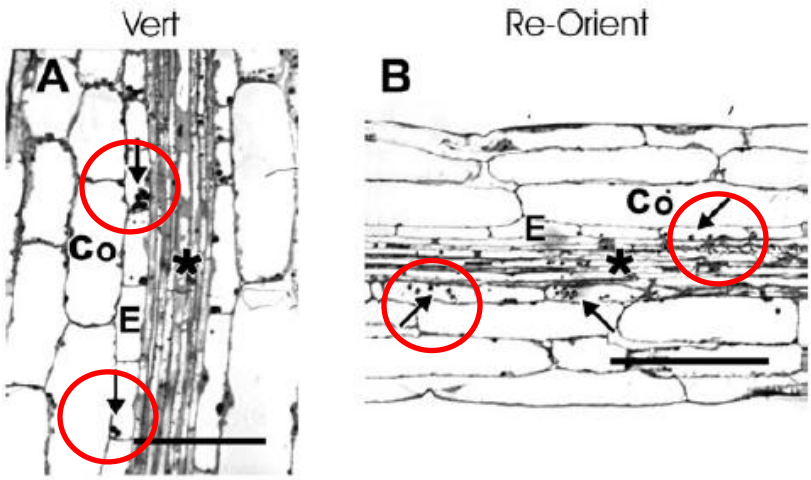

Figure 6.10 – Light microscopy images of Arabidopsis shoots before and after 90° reorientation. The endodermal layer is labeled E, the cortex is labeled Co, and the vascular tissues are marked with an asterisk. The arrows/circles show the amyloplasts within the endodermis [49]. Bar = 25μm.

In Figure 6.8, an Arabidopsis thaliana plant is shown vertically oriented (a) and then placed horizontally (b) with the power of microscopic imaging [44]. In the vertical shoot (a), the starch-filled amyloplasts are found at the bottom of the endodermal cells due to sedimentation. However, the lateral orientation for each layer is symmetric and curvature is not induced. Cells in (b) are in the horizontal direction with the image being shot immediately after reorientation [45]. The amyloplasts are depicted moving from the former base to the new bottom of the endodermis. This new sedimentation results in gravitropic curvature. Horizontal plant stems exhibit the most curvature because they are always reorienting in the direction of gravity.

In terms of establishing a model, the vertical position will be the point in which the initial amount of auxin a_0^a will be unaffected. Figure 6.9 exhibits the different stem movements of an

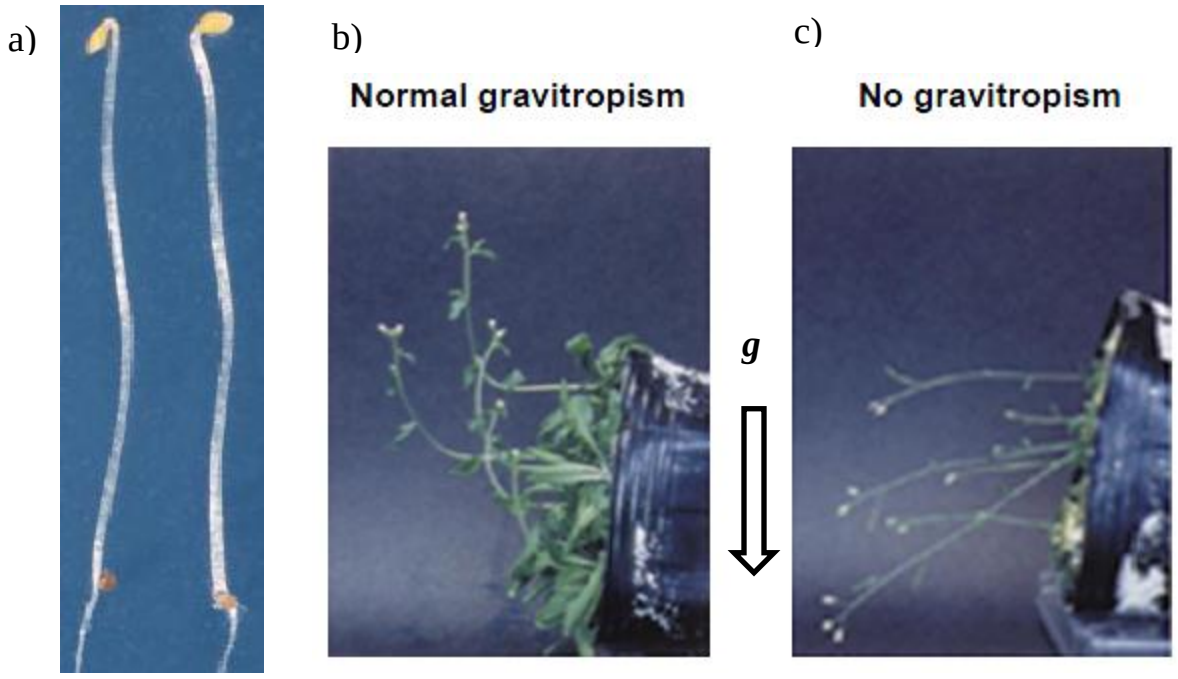

Figure 6.11 – Various orientations of Arabidopsis thaliana plants are presented under different circumstances. Young seedlings in (a) are grown vertically in darkness, horizontal seedlings (b) show curvature against the gravity vector, and a mutated plant (c) with no endodermis has no curvature [45, 51].

Arabidopsis thaliana plant with different positions. Young seedlings in (a) were grown in an environment that only had gravity as the stimulus [45]. They have grown straight up against the direction of gravity and show very little to zero curvature. Amyloplasts are moving at the cellular level but since the orientation is symmetric, large curvature is not apparent. For the model, this defines $a_{,Y}$ as zero which is an important boundary condition. Next, the greatest curvature is found at a horizontal orientation as shown by Figure 6.9b. The horizontal position means that there is an auxin gradient and it is at its maximum. $a_{,Y}$ is no longer zero and is at its greatest when horizontally oriented. A defining angle is going to be essential to orientation and the equation for relating auxin to gravity.

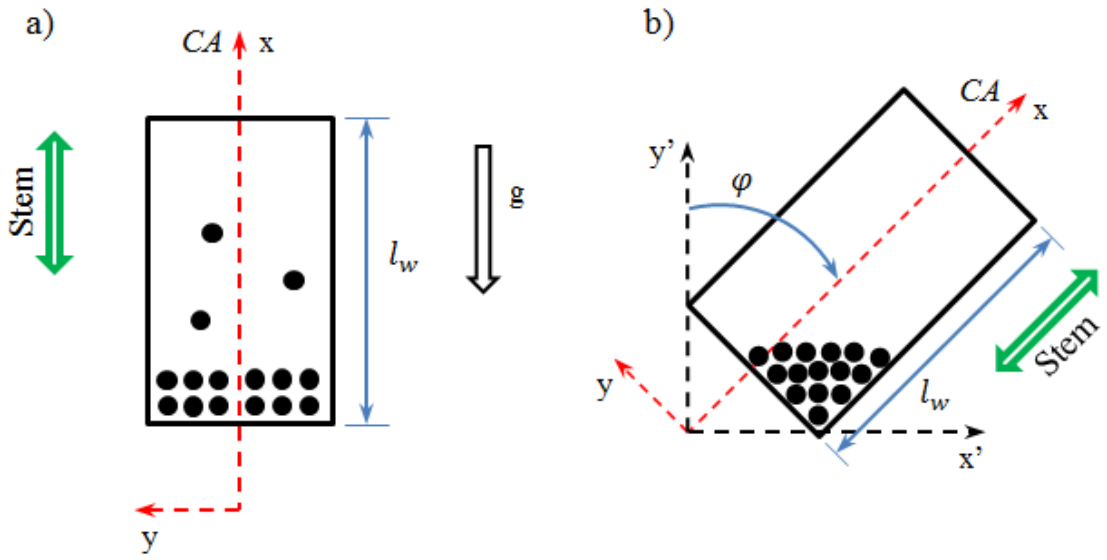

Figure 6.12 – A single cell mockup of endodermal cells vertically oriented (a) and angled at a certain angle (b). The direction of the stem and gravity are noted. φ is the angle between the vertical and the newly oriented cell. The black dots are the amyloplasts that sediment due to gravity.

With the aid of Figure 6.10, the concept behind the equation for gravitropism as a function of auxin can be better comprehended. The role of amyloplasts in compiling a function for auxin movement is important for gravitropsim. In the vertically oriented stem, the base of the cell is where the amyloplasts will settle and curvature will not occur, $a_{,Y} = 0$. It is when the plant is not in a vertical orientation that the starch filled organelles will begin to sediment on the lateral walls l_w as shown in Figure 6.10b. At a fully horizontal orientation, $\varphi = \pm\frac{\pi}{2}$, amyloplasts will settle along the entire length of l_w. Therefore, a key component to the equation will be the ratio of the wall length l_w at angle φ compared to the entire wall length l_w at complete horizontal orientation. Geometric analysis defines that ratio as $\frac{l_w \sin(\varphi)}{l_w}$ which accounts for amyloplast movement. Thus, the following equation defines the movement of auxin across the cell

$$a_{,Y}\,(x, y) = a_0(x) * \sin(\varphi) \tag{6.36}$$

where φ is the angle between the vertical in the upper quadrants and the centroidal axis *CA*. Equation 6.36 satisfies the boundary conditions since it will equal zero when φ equals 0° and it will be at its greatest when φ equals ±90°. The main equations can now be completed and experimental data can be used.

6.7 The Final Equations

6.7.1 Assembly of Macro Functions

Through engineering derivation and data formulation the crux of the model can be finally assembled with some backtracking. Using Equations 6.24 and 6.35, the complete equation for auxin movement due to phototropism is

$$a(x,y) = a_0(x) + \xi\big(I_2(x) - I_1(x)\big) * \cos(\varphi) * y \tag{6.37}$$

The combination of Equations 6.24 and 6.33 result in the gravitropic function

$$a(x,y) = a_0(x) + a_0(x) * \sin(\varphi) * y \tag{6.38}$$

Now that equations for auxin have been formulated, Equation 6.22 can be revamped with the inclusion of Equations 6.26 and 6.30 to finally look like

$$\frac{du}{dx} = \varepsilon_0(x) = \lambda[\bar{C}(x) + \alpha a_0^a(x)] * [\bar{P}(x) + \beta a_0(x)]$$

$$= \lambda * \bar{C}(x) * \bar{P}(x) + \lambda\beta * \bar{C}(x) * a_0(x) + \lambda\alpha * \bar{P}(x) * a_0(x) + \lambda\alpha\beta * a_0(x)^2 \tag{6.39}$$

This equation is for the axial strain within the shoot and is dependent on constant values at the initial setting of the plant. λ, β, and α are constants based off the stress-pressure relationship, the auxin-turgor pressure linear relationship, and the auxin-compliance linear relationship respectively. $\bar{C}$ and $\bar{P}$ are the initial values of compliance and turgor pressure before the onset of tropisms. Lastly, a_0 is the initial amount of auxin within the plant before it responds to stimuli.

Curvature is the final equation that is put together. The introduction of Equations 6.27 and 6.31 transform Equation 6.21 into

$$k(x,y) = \frac{d\varphi}{dx} = -\lambda([\bar{C}(x) + \alpha a_0(x)] * \beta a_{,Y}(y) + \alpha a_{,Y}(y) * [\bar{P}(x) + \beta a_0(x)])$$

$$= -\lambda[\beta * \bar{C}(x) + \alpha * \bar{P}(x) + 2\alpha\beta * a_0(x)] * a_{,Y} \quad (6.40)$$

The only unknown in this equation is $a_{,Y}$ and that is dependent on the tropism being introduced to the plant. Equations 6.35 and 6.36 are for phototropism and gravitropism, respectively.

6.7.2 Integration of Equations

Now that the macro equations have been reworked and compiled, the next step is to integrate to determine the equations for slope φ with respect to each tropism. In the case of phototropism, Equations 6.19, 6.35, and simplification of Equation 6.40 results in the following differential equation

$$\frac{d\varphi}{dx} = -\lambda[\beta * \bar{C}(x) + \alpha * \bar{P}(x) + 2\alpha\beta * a_0(x)] * \xi\big(I_2(x) - I_1(x)\big) * \cos(\varphi)$$

$$\frac{d\varphi}{dx} = G * \cos(\varphi)$$

$$\frac{d\varphi}{dx} - G * \cos(\varphi) = 0 \quad (6.41)$$

where G is equal $-\lambda[\beta * \bar{C}(x) + \alpha * \bar{P}(x) + 2\alpha\beta * a_0(x)] * \xi\big(I_2(x) - I_1(x)\big)$. An important boundary condition for a vertically oriented shoot is at the base of it where $\varphi(0) = 0$. Together with this boundary condition and some mathematical massaging the integral can be taken as

$$\left[\frac{d\varphi}{dx} - G * \cos(\varphi)\right]\frac{dx}{\cos(\varphi)} = 0$$

$$\int_0^{\varphi} \frac{d\varphi}{\cos(\varphi)} = \int_0^{x} G dx \quad (6.42)$$

After integration and solving for φ, the equation for slope with respect to phototropism is

$$\varphi_{PHOT}(x) = 2\arctan(e^{Gx}) - \frac{\pi}{2} \tag{6.43}$$

The final step is to find deflection *v* through the integration Equation 6.43 but standard integration will provide an unwanted headache. Thus, numerical integration with the power of MATLAB will allow for great assistance.

Similar to phototropism, the determination of $\varphi(x)$ will follow the same approach for gravitropism. The differential equation for gravitropism combined with Equations 6.19, 6.36, and 6.40 will be

$$\frac{d\varphi}{dx} = -\lambda[\beta * \bar{C}(x) + \alpha * \bar{P}(x) + 2\alpha\beta * a_0(x)] * a_0(x) * \sin(\varphi)$$

$$\frac{d\varphi}{dx} = G * \sin(\varphi)$$

$$\frac{d\varphi}{dx} - G * \sin(\varphi) = 0 \tag{6.44}$$

where G is equal to $-\lambda[\beta * \bar{C}(x) + \alpha * \bar{P}(x) + 2\alpha\beta * a_0(x)] * a_0(x)$. For a horizontally oriented shoot, the boundary condition at the base of the shoot is $\varphi(0) = \frac{\pi}{2}$. Further mathematical manipulation and the utilization of the boundary condition produces

$$\left[\frac{d\varphi}{dx} - G * \sin(\varphi)\right]\frac{dx}{\sin(\varphi)} = 0$$

$$\int_{\frac{\pi}{2}}^{\varphi} \frac{d\varphi}{\sin(\varphi)} = \int_0^x G dx \tag{6.45}$$

The integration of Equation 6.45 results in the following equation for gravitropism

$$\varphi_{GRAV}(x) = 2\arctan\left(\tan\left(\frac{\pi}{4}\right) * e^{-Gx}\right) \tag{6.46}$$

Once again, the final procedure is to integrate Equation 6.46 to find the deflection that the shoot will undergo when the gravity vector is recorded in the plant.

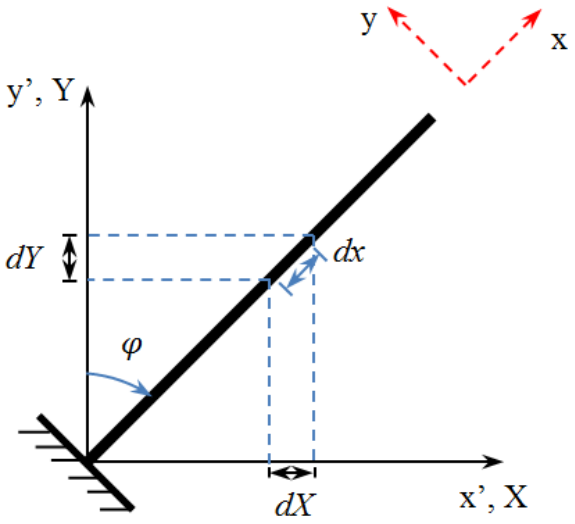

Figure 6.13 – A plant stem breakdown used for the model.

With Figure 6.13, trigonometric correlations are fortified and established as shown through Equations 6.47 and 6.48.

$$\frac{dY}{dx} = \cos(\varphi) \tag{6.47}$$

$$\frac{dX}{dx} = \sin(\varphi) \tag{6.48}$$

The integration of Equations 6.47 and 6.48 will lead to the deflection of the plant shoot and will provide a curvature response. In Equations 6.49 and 6.50, the integration of *dY* and *dX* are shown.

$$Y = \int_0^x \cos(\varphi)\, dx \tag{6.49}$$

$$X = \int_0^x \sin(\varphi) dx \tag{6.50}$$

Equations 6.43, 6.46, 6.49, and 6.50 will be integrated numerically with the power of MATLAB. The model has now been completed and the procedure of investigating constant values with model implementation is the next step.

Chapter Seven

Model Implementation and Investigation

7.1 Benchmark Problems

7.1.1 Values Associated with Constants

Completion of the model has now given way to examining the different variables that are taken into account. There are a number of constants that the model relies on and it is vital to understand how they affect constraints. Equation 6.40 is associated with the curvature that the shoot will experience is dependent on multiple initial conditions. λ is the constant that relates pressure and stress within the cell since it is equal to $\frac{R}{2t}$. In terms of auxin and turgor pressure, β and $\bar{P}$ are important since they provide a much needed linear relationship. α and $\bar{C}$ are constants that integrate auxin and compliance into the model. ξ is a constant involved with the relationship of light intensity. In the model it will have an assumed value of 1.0 since experimental data could not be found. Initial auxin a_0 found in the shoot is another crucial piece to the puzzle.

Each of these constants will play a role in affecting the overall deflection the shoot undergoes. In Table 7.1, values that are associated with each constant have been found through rigorous data research. These values will be used in formulating the model and examining benchmark problems. Plant experimentation and research has been a field of growing interest with advances in technology catering better results. However, the values used in this model are experimental and will be further examined.

Table 7.1 – Range of values that are associated with model constants.

Parameter	Definition	Values	Value Used	Source
R	Cell radius	5 – 50μm	20μm	[54, 70]
t	Cell wall thickness	0.1 – 10μm	5.0μm	[55]
$\bar{P}$	Initial turgor pressure	0.4 – 0.85MPa	0.60MPa	[56, 71]
E	Young's Modulus	21.3 – 27.5MPa	24.4MPa	[57]
$\bar{C}$	Initial compliance	0.0364 – 0.0469MPa^{-1}	.0417MPa^{-1}	[57]
a_0	Initial auxin concentration	0.4 – 4.0μM	2.2 μM	[58]
β	Turgor Pressure vs. Auxin	3.13MPa/μM	3.13MPa/μM	[59]
α	Compliance vs. Auxin	.024$MPa^{-1}\mu M^{-1}$	.024$MPa^{-1}\mu M^{-1}$	[60]

Table 7.2 – Distinction of plants used for the research values found in Table 7.1

Parameter	Plant Type	Common Name	Source
R	Pea, Oat, Yeast, etc	Pea, Oat, Yeast, etc	[54, 70]
t	Pea, Oat, Yeast, etc	Pea, Oat, Yeast, etc	[55]
$\bar{P}$	Pisum sativum, Yeast	Pea, Yeast	[56, 71]
E	Arabidopsis thaliana	Thale Cress	[57]
$\bar{C}$	Arabidopsis thaliana	Thale Cress	[57]
a_0	Avena	Oat	[58]
β	Pinus Radiata	Monterey Pine	[59]
α	Vigna Unguiculata	Cow Pea	[60]

The cell radius values were taken from cells found in various plants ranging from the smallest cell types to the largest [54, 70]. In terms of cell wall thickness, the value was found examining various plant cells and taking an accepted average [55]. The initial turgor pressure value was derived from the in depth research done with pea stems and fungal plants [56]. Young's modulus and compliance values were taken from testing done with Arabidopsis thaliana stems [57]. Values used for initial auxin concentration in Table 7.1 came from Avena, or oat plant, research [58]. The turgor pressure-auxin relationship was acquired with testing that involved Radiata plants while the compliance-auxin value was extracted from Vigna Unguiculata experimentation [60]. The value for ξ was taken as $1cm^2$ since research for the area to auxin relationship was very limited.

7.1.2 Phototropism and the Model

The first set of benchmark problems to test the model involves a vertical shoot orientation under the effect of phototropism. In the first case, the shoot will be vertically oriented which is properly labeled and depicted in Figure 7.1.

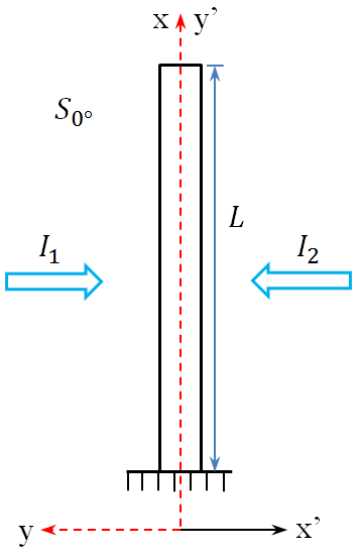

Figure 7.1 – A vertical shoot used to test the model.

As stated previously, during the process of phototropism the plant will reorient itself in the direction of light. In the case of the model, when $I_2 > I_1$ the stem should bend to the right and when the difference is reversed, it should bend to the left. The value of L used was 11cm and was taken from personal measurements of Isis Candy tomato seedlings. Light intensity values varied from 1 to zero for both values of I [58].

After running the model, the stem response was correct for each scenario. However, the results did not model the proper response of plants in the actual environment. With this benchmark problem, the model is shown to properly bend in the direction of greatest light. MATLAB was used to numerically integrate the function of φ and graphed the results. A

working model in terms of phototropic response has been established however, the deflection of the shoot is much greater than what is expected. Further analysis of the model will be discussed later.

7.1.3 The Model in Terms of Gravitropism

Unlike phototropism, a plant bends against the direction of the gravity vector that has been perceived. The orientation of the plant is an integral part in gravitropism as well as overall plant sustainability. A vertical plant stem will not have any gravitropic curvature since it is in the same direction of the gravity vector. It is when the plant is positioned in the horizontal direction that a gravitropic response will take place. Two benchmark problems that are used in testing the model are a completely horizontal stem and a stem that is oriented at 45°. Figure 7.2 shows the intended orientation of the different stems that will be used in the model.

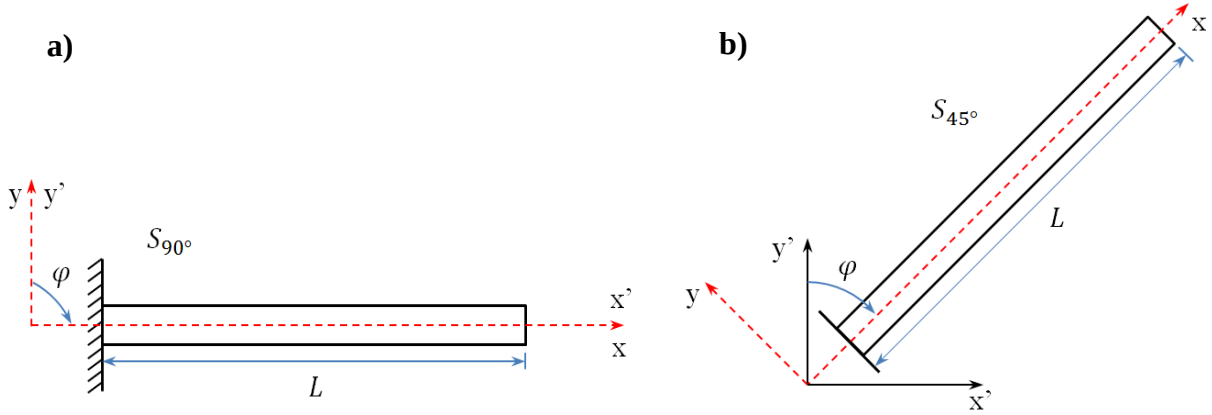

Figure 7.2 – A plant with two orientations that are used to test the model being implemented for gravitropic response. A completely horizontal stem (a) and one that is oriented at 45° (b).

Similar to phototropism, the model will use the same values found in Table 7.1 and the same length *L*. Both cases should show upward curvature since the stem is trying to reorient itself in the vertical direction against the vector of gravity.

Once again the model displays the proper response in both shoot orientations. However, the amount of curvature is far too drastic similar to the case of phototropism in the model. The process and function behind the model is correct but a reevaluation of the constant values is needed. Comparison with experiments will reveal which values are creating such a huge response.

7.2 Testing the Model with Data from Research

7.2.1 Model Overview and Tropisms

The established model is effective in simulating the proper curvature response with both phototropism and gravitropism. Yet, the degree in which the curvature of the stem occurs does not accurately reflect the in situ response. Therefore, an examination of the research values found in Table 7.1 is necessary. Also, a comparison with data taken from personal experimentation provides another avenue to improve the model. A concentration on gravitropism was utilized since eliminating all other factors that can attribute to plant curvature was an important part of the experiment. Phototropism involves the varying of light intensity but the factor of gravity still remains. To eliminate gravity, testing would have to be done out in space or in a zero gravity vessel. In either case, the possibility for personal testing was not considered. To test for gravitropism, the absence of light is needed and creating such an atmosphere was simple.

7.2.2 Model and Research Data Interaction

The first constant to be examined is the powerful plant hormone auxin. It is a naturally occurring substance that brings about the curvature of the plant. In gravitropism, the plant senses the gravity vector through the endodermis and sends a signal that activates cells in which auxin laterally moves to. In the case of the horizontal plant shoot, auxin will move to the side that is closest to the ground thus allowing the plant stem to curve upwards against gravity. The following model interaction accounts only for pure gravitropism (i.e. self-weight not accounted for).

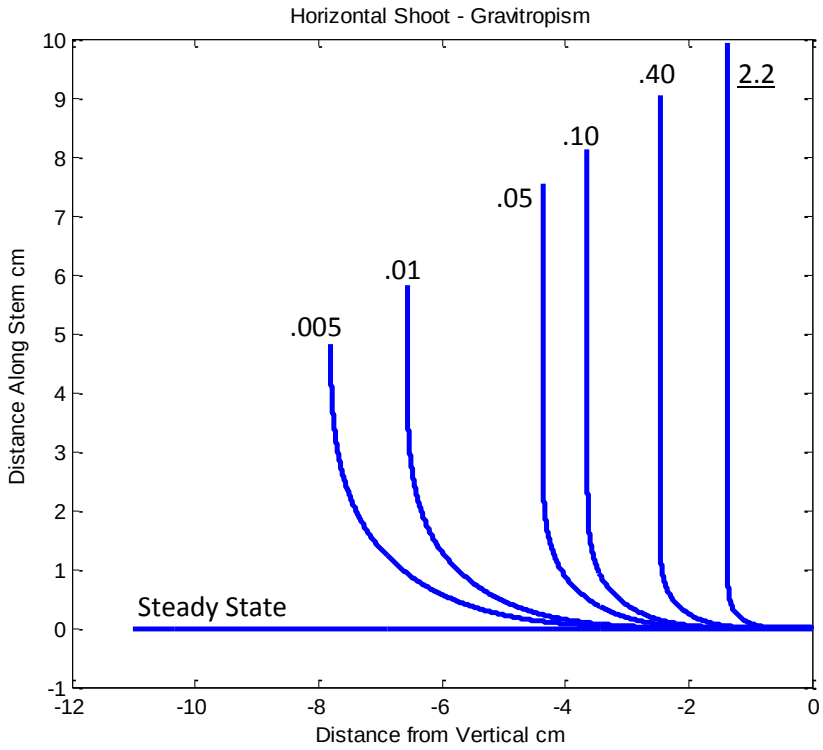

Figure 7.3 – Various plant shoot orientations with changing values of initial auxin concentration. The initial value of auxin used is underlined. Auxin is measured in μM.

In Figure 7.3, the value of initial auxin changes from 0.005-2.2μm and the orientations are significantly altered. As auxin increases, the curvature in the shoot increases rapidly and from

research this relationship holds true. The initial value of 2.2μm appears to be too high of a value while the auxin concentration of 0.01μm renders a more environmental response similar to an actual plant. The next constant to be adjusted is the initial compliance $\bar{C}$ of the cells within the shoot.

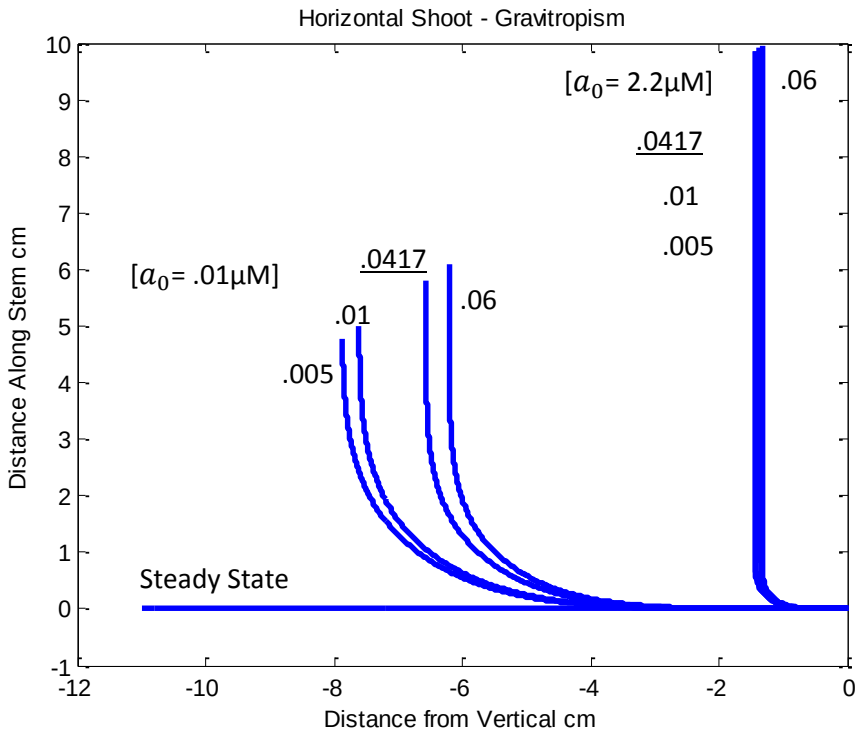

Figure 7.4 – Different orientations of the plant shoot with changing values of initial compliance $\bar{C}$. The first set considered all the constant values used in Table 7.1 while the second set took the new auxin concentration found with the model. Compliance is measured in MPa^{-1}.

The effect of varying the initial compliance is shown in Figure 7.4. The first set of curves deals with the set of values first used in Table 7.1 while the second set of curves takes into account the new initial auxin concentration found with model manipulation. The first set of curves cannot be distinguished because the level of auxin overtakes any change to compliance. Therefore, the reduced value of auxin in the second set is a better match for actual plant response. Compliance deals with the stiffness of the cell walls and as it increases the curvature within the plant

increases as well. An increase in compliance allows the cell to expand with the uptake of water and increase of turgor pressure. The value of .01MPa^{-1} illustrates the closest curvature to plants out in the environment and will be used for the rest of the model interrogation. The next value to be tested is the relationship between compliance and auxin concentration α.

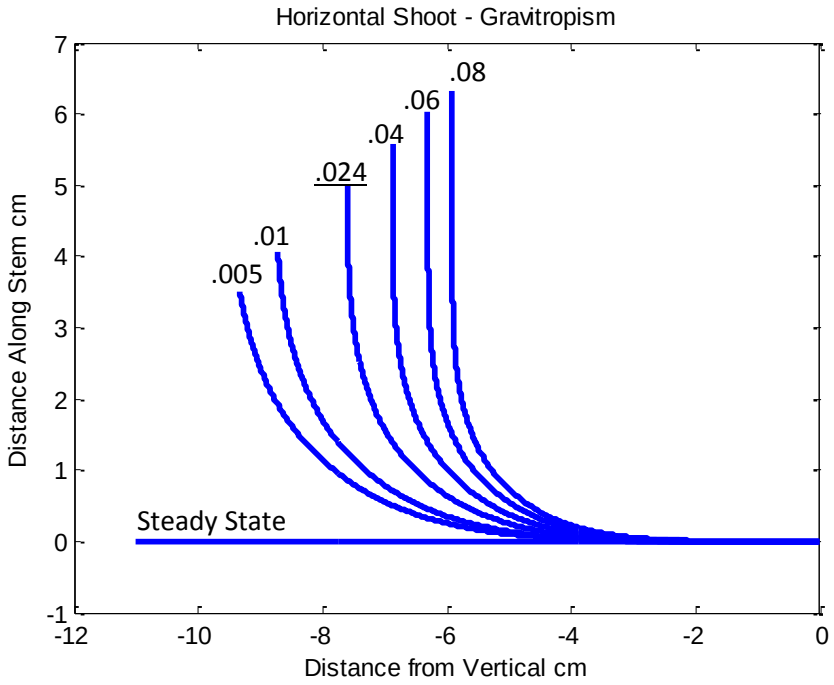

Figure 7.5 – The change in shoot orientation is due to the different values of α (MPa^{-1}μM^{-1}) utilized by the model. Initial α used is underlined.

With changes in the compliance-auxin relationship constant, the plant stem varies slightly from the original initial value of .024MPa^{-1}μm^{-1}. However, from Figure 7.5 it appears that .024MPa^{-1}μM^{-1} provides a more realistic approach to plant curvature. α is assumed to be a linear relationship between compliance and auxin concentration. When both compliance and auxin increase the curvature increases as well. The value of .024MPa^{-1}μM^{-1} will be kept the same and initial turgor pressure is the next constant to undergo examination.

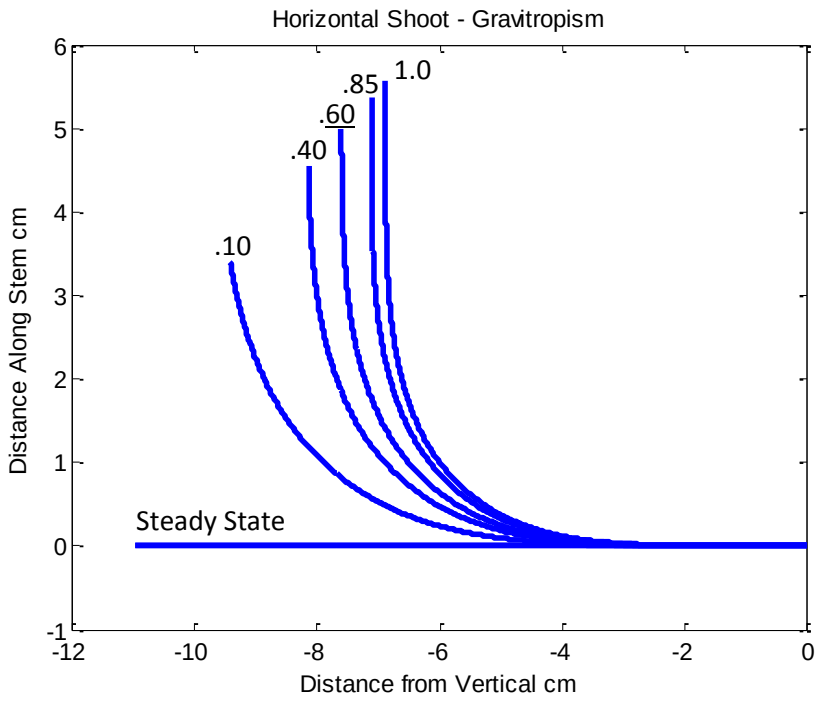

Figure 7.6 – Initial internal turgor pressure $\bar{P}$ is modified in the model and produces the various changes in plant stem deflection. Initial value for pressure is underlined.

Results from the change in initial turgor pressure $\bar{P}$ are displayed in Figure 7.6. Turgor pressure involves the uptake of water via the vacuoles within the cytoplasm of the cell. As pressure increases, indicated in the above figure, the curvature of the shoot increases and vice versa. The value for initial turgor pressure, 0.4MPa, provides the best curve option for the model. Although curvature seems to be getting better, the model seems to be lacking in providing a realistic curvature that is associated with an in situ plant. This could potentially be associated with some of the linear assumptions taken into account for the model. The last constant to be reviewed is the relationship β between turgor pressure and the concentration of auxin.

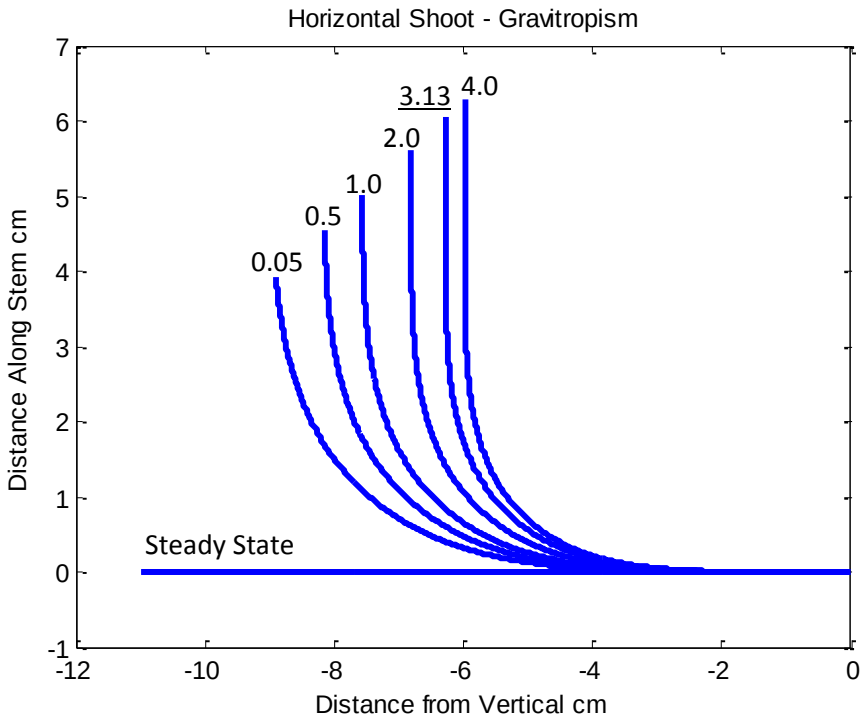

Figure 7.7 – The modification of the value β (MPa/μM) in the model demonstrates the change in orientation of the plant stem.

Turgor pressure and auxin concentration are significant factors when dealing with overall plant curvature. At the molecular level, auxin activates proton pumps that inform the cell to increase its uptake of water therefore increasing the turgor pressure within the cell. Hence, the increase of turgor pressure can be directly related to the increase of auxin located at the cellular level. For the model, a linear relationship was assumed for β and Figure 7.7 reveals that the experimental value first used was a bit large. At 0.5MPa/μM, the deflection of the horizontal plant shoot is a bit closer to agreeing with the observed plant curvature. Overall, the model has improved with a much more realistic deflection and is within the same realm of gravitropic curvature that can be found with plants out in their natural habitats.

7.2.3 Comparison of Results

The overall curvature response in the model is effective in capturing how a plant shoot reorients itself in its own environment. The initial values found from research and previous experiments contributed to an effective starting point in terms of model formulation. Once benchmark problems were tested, the constants first used were able to be examined. Implementation of the model demonstrated that such values were close but not accurate enough. Examination of the important constants found in Table 7.1, the model could be reevaluated and improved. Table 7.3 differentiates between the initial values used and the values that corresponded to a better curvature response. Compliance and pressure values changed but only marginally, which proves that the model and research data are in similar agreement. The relationship of α was on point but it was the initial value of auxin and β that had a major effect on the model. Such a difference truly shows the power of the plant hormone and the control it has with phototropism and gravitropism.

Table 7.3 – A comparison of values that were initially used and the change in values with the utilization of the model.

Parameter	Initial Experimental Value	Adjusted Value
$\bar{P}$	0.60MPa	0.40MPa
$\bar{C}$	.0417MPa^{-1}	.010MPa^{-1}
a_0	2.2μM	.010μM
β	3.13MPa/μM	0.50MPa/μM
α	.024MPa^{-1}μM^{-1}	.024MPa^{-1}μM^{-1}

7.2.4 Gravitropism: Testing and Experimental Data

The process behind testing for gravitropic response involved the elimination of all variables except the gravity vector. First, a 34.5x26.9x28.4cm cardboard box was furnished with black construction paper to eliminate any presence of light. A 23.9x10.2cm rectangle was cut out from the larger side for easy access to take pictures as shown in Figure 7.8. A Canon EOS Rebel XS digital camera with a mini tripod was placed 24cm away from the rectangular cut out. Isis Candy tomato seedlings purchased from Harlequin's Garden in Boulder, CO were placed

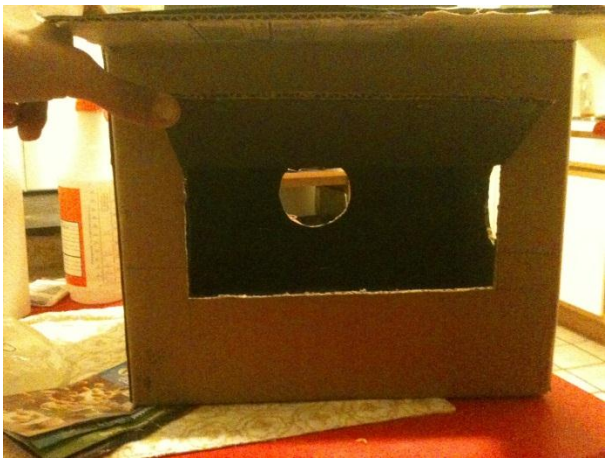

Figure 7.8 – The cardboard box with black construction paper on the inner walls used for gravitropic testing.

horizontally within the box. Extra pieces of black construction paper were used to prevent any trace of light from entering the box. The average length of the tomato seedlings from the top of the soil to the stem apex was 11cm. Pictures were taken at twenty minute intervals over a four hour period of time. Measurements were from the horizontal axis to the plant stem were taken. From the group of plants, I chose the plant with the best curvature response shown in Figure 7.9. In Table 7.4, values from the gravitropic curvature where obtained from the measurements of

deflection. This data will be used to examine the various constants that go into the model for gravitropism.

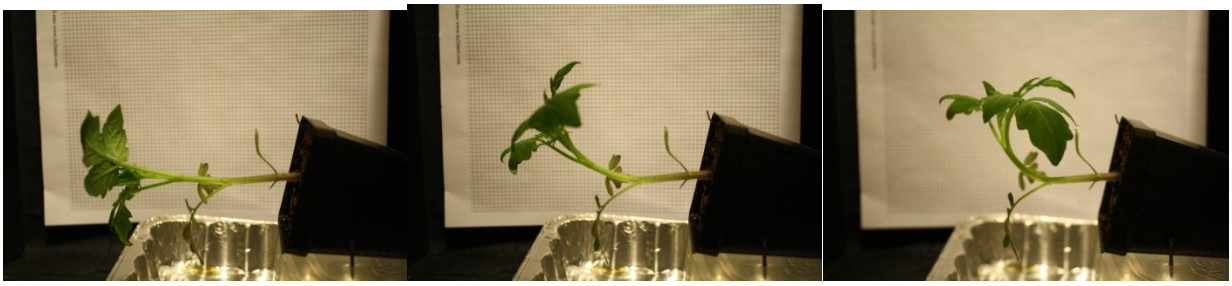

Figure 7.9 – The plant responding to the gravity vector over a period of 4 hours.

Table 7.4 – Values associated with gravitropic response from personal testing

X-Axis (cm)	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	6.9	7.1
Y-Axis (cm)	0	0	0	0	0	0	-0.1	-0.2	-0.2	-0.1	0.1	0.4	0.9	1.7	2.8	3.5

7.2.5 Model and Experiment Interaction

A model is a key component in producing a link between research and data acquired from testing. The model that has been established is effective in reproducing the proper response to both phototropism and gravitropsim. In the model, the various constants found in Table 7.1 have been tested and new values in Table 7.3 have been implemented. The addition of the experimental data in Table 7.4 will provide a curve for the model to match. Those values in Table 7.3 will be manipulated to try and fit the experimental data as best as possible.

Figure 7.10 depicts the initial condition that is used for the model since it closely resembles the orientation of the Isis Candy tomato seedling that was used during testing.

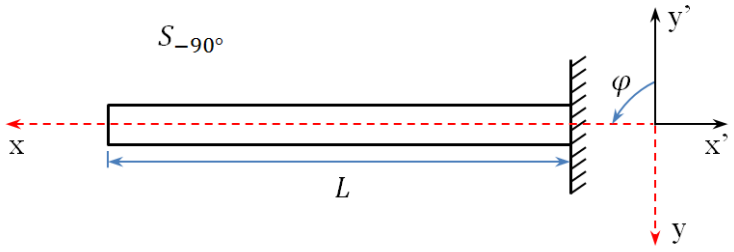

Figure 7.10 – The orientation of the stem used in the model for comparison of plant testing.

Figure 7.11 illustrates the differences between the model without accounting for self-weight and the experimental data found through testing. There is a disparity in the magnitude of the response between the model and in situ experimentation. A correction to the model must be made since the only force being considered is the turgor pressure within the cells of the stem.

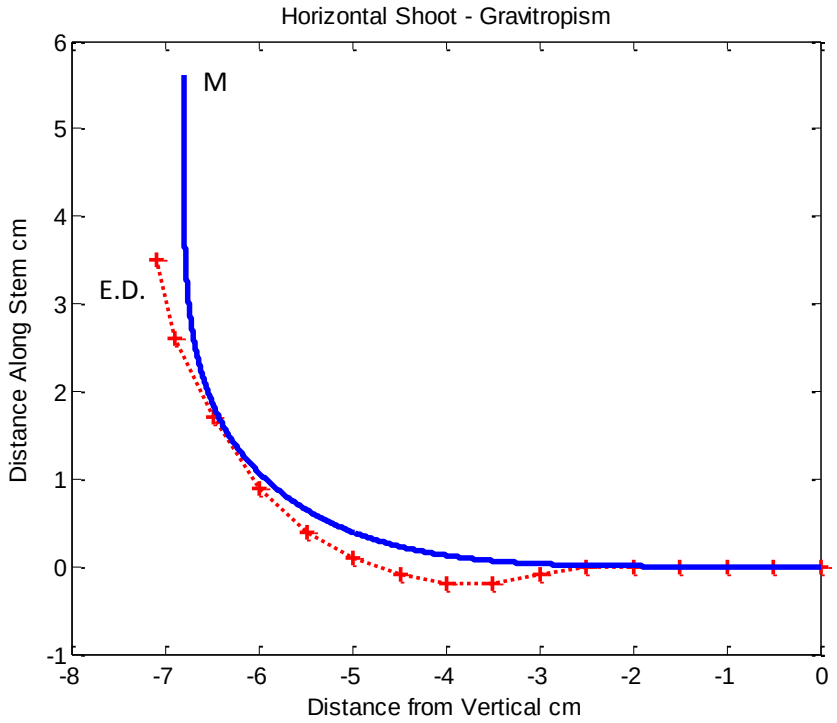

Figure 7.11 – Experimental data (E.D.) is graphed against the model (M), which only accounts for pure gravitropsim.

7.3 The Addition of the Gravity Load

A comparison with the experimental data still illustrated a large difference between 3cm and 6cm of the plant shoot, which can be seen in Figures 7.11. After reviewing the model, the self-weight of the stem was not taken into consideration since the only force the model utilized was the turgor pressure. To take the self-weight of the stem, beam deflection theory was used to derive functions for deflection [53]. At first, a uniformly distributed load case was considered for the plant shoot. However, the equations for deflection did not mesh with the model since they were for small deflections. Another approach was taken to correct the model with the addition of the self-weight.

In the model, values for φ_{GRAV} were calculated using the values found in Table 7.1. Then, the integration of φ_{GRAV} was initiated by using Equation 6.46 and finding the area below the curve that was created. The deflected shape of the plant shoot was taken as the initial curved shape of the shoot. Uniform loads were placed between each node of the shoot that was created through the model. Starting from the end of the shoot, moment was calculated by multiplying the uniform loads by the distance between the nodes. The total moment was calculated for the entire shoot and the value for curvature was determined by dividing moment by Young's Modulus and moment of inertia. The integration of curvature led to the slope due to the weight of the shoot. This new slope was added to φ_{GRAV} and the total deflection due to self-weight and gravitropism was calculated. A new deflection curve was revealed demonstrating a more realistic curvature response.

Essentially, deflection due to gravity is found and that configuration becomes the initial shoot curvature. This is followed by applying a uniform load associated with the plant self-weight at each node of the shoot. Moment is calculated and curvature due to self-weight is found.

Moment is integrated and added to the model. A new φ is determined and integrated again resulting in a deflected shoot shape that takes into account both self-weight and gravitropism. The values for self-weight and Young's Modulus for the stem were taken from experimental data. Self-weight was equal to 27mg/cm and 140MPa was used for the modulus [69, 72]. Figure 7.12 depicts the difference of deflection between gravitropism, gravitropism with the addition of self-weight, and the experimental data using the values in Table 7.3

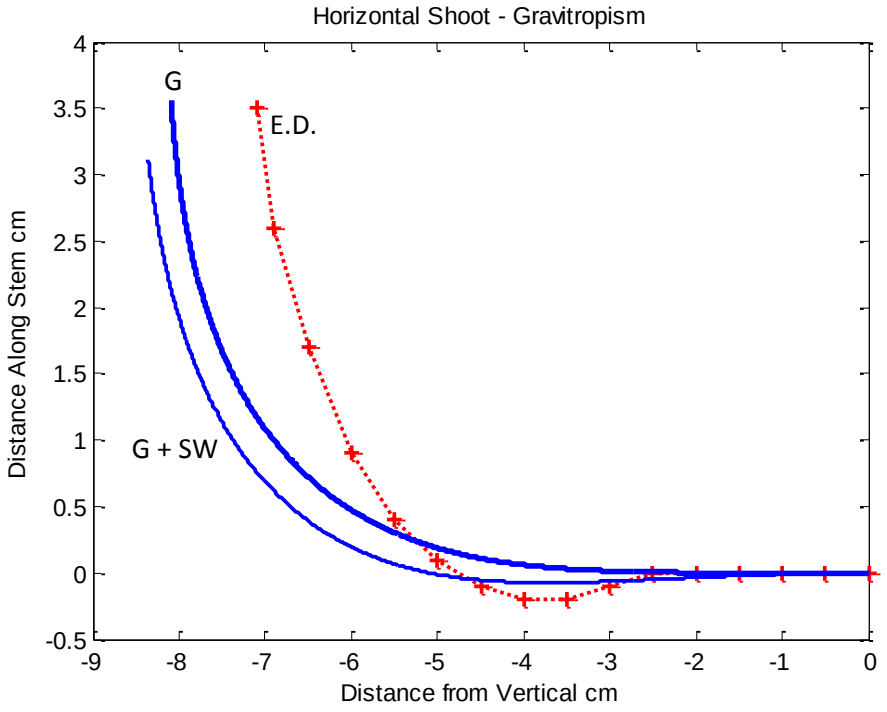

Figure 7.12 – A comparison of deflection for pure gravitropism (G), gravitropism and self-weight (G + SW), and the experimental data (E.D.).

As shown in Figure 7.12, the addition of the gravity load acts as a correction factor that provides a more plant like response. Overall, the response is effective but there is still a noticeable difference between the model and experimental data. Gravitropism with the addition

of the gravity vector will be further examined. First, the self-weight will be kept constant and changes in auxin will be made to produce a closer model curvature response.

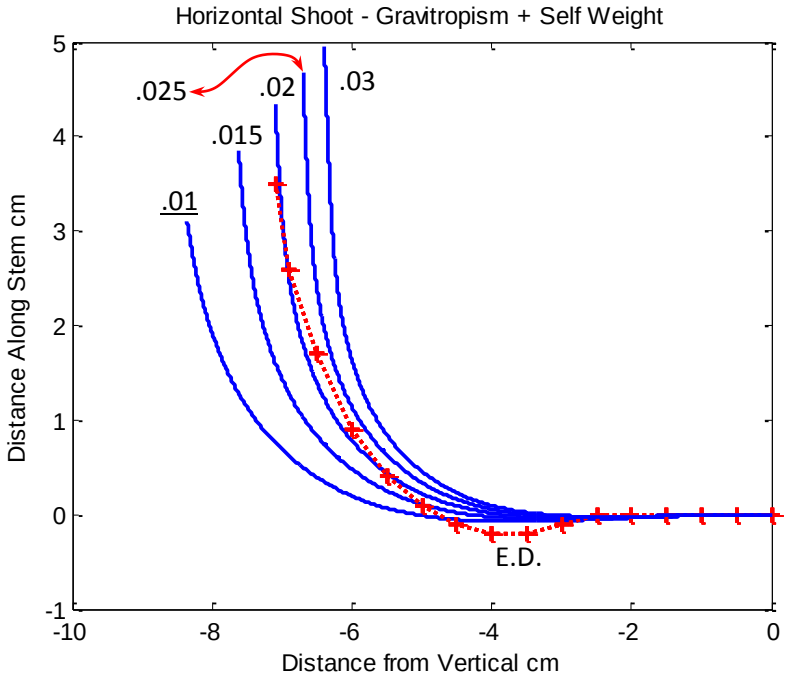

Figure 7.13 – Model data of gravitropism and self-weight are compared with experimental data. The value from Table 7.3 for auxin is underlined. Auxin concentration is measured in μM.

In Figure 7.13, the variation of auxin is shown using the same values in Table 7.3 and a self-weight of 27mg/cm for the shoot. As auxin increases, the curvature response of the shoot increases as well. This is a key relationship of the model that has been retained throughout testing. The best fit curve from the above graph is when auxin is equal to 0.02μM with a much smaller difference between model and experimental data. The next step is to keep the same values in Table 7.3 but change the self-weight of the stem.

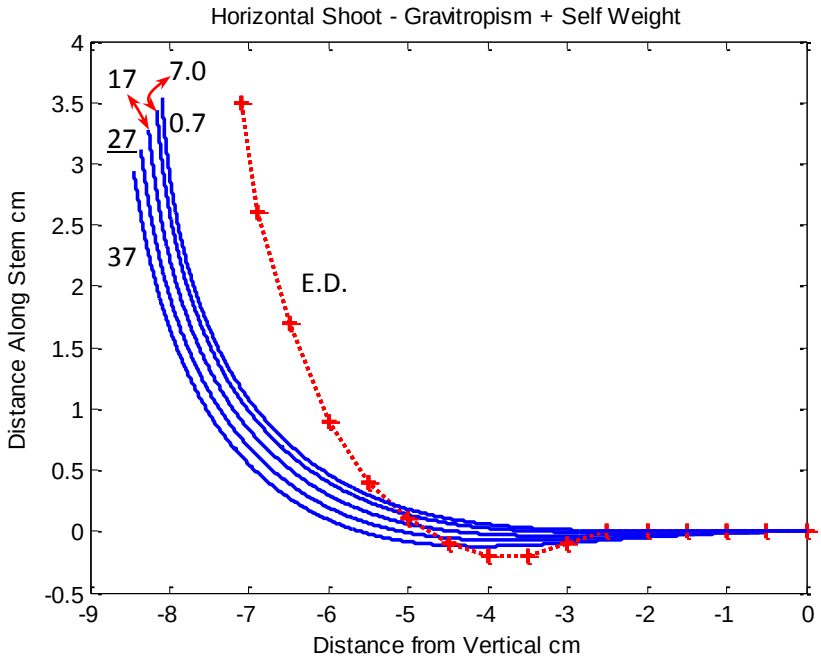

Figure 7.14 – Self-weight (mg/cm) of the stem is manipulated and compared with experimental data (E.D.). The initial value of self-weight used is underlined.

As self-weight changes, Figure 7.14 illustrates how it affects the gravitropic curvature response of the shoot. The lower the self-weight the more upward curvature it is going to have since auxin is still playing a role and dictating deflection. There is still a large difference between the model and the experimental data used from Table 7.4. Self-weight is a large factor when considering the correction of the gravitropic response however it seems that there is a relationship between self-weight and auxin that must be analyzed. The final step is to produce a curvature response based on changes to both auxin and self-weight of the shoot.

Competition between auxin concentration and self-weight of the plant shoot plays a major role in establishing a curvature response that is indicative of an in situ environment. Utilizing this relationship, values for auxin and self-weight were varied until the best curvature, within reason, for the model matched the experimental data. In Figure 7.15, the model is shown to have the smallest difference with respect to the curvature found from gravitropic testing.

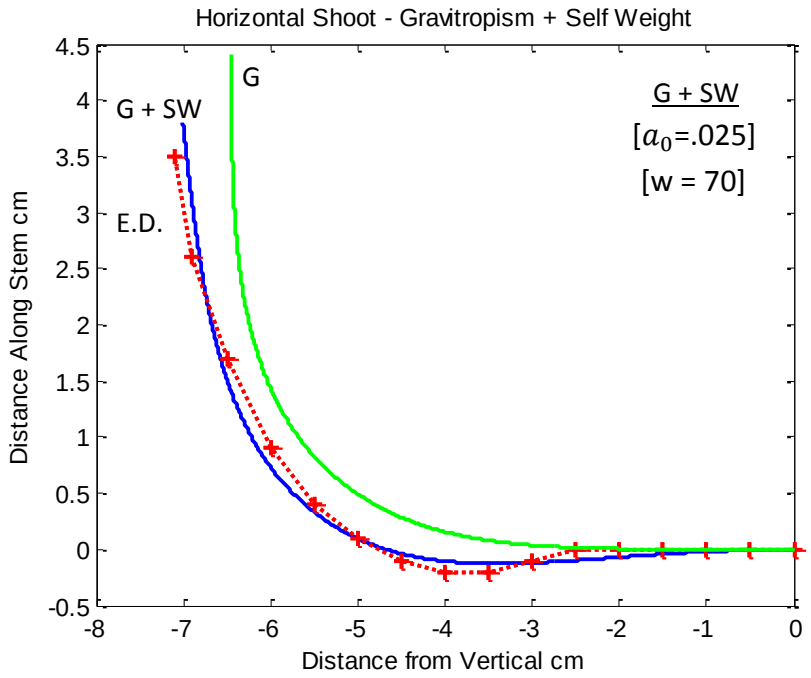

Figure 7.15 – Best fit curve from the model with initial auxin concentration of .025μM and self-weight of 70mg/cm compared to experimental data (E.D.) and pure gravitropism (G).

Initial auxin concentration and self-weight compete with each other in the model to emulate the best gravitropic curvature response. Auxin has more of a control over the system than self-weight because it is the defining element for tropism and plant curvature. To maintain the curve in Figure 7.15, auxin concentration was increased from .01μM to .025μM while self-weight increased from 27mg/cm to 50mg/cm. Also, Figure 7.14 depicts minor changes with large increments of self-weight therefore adding to the importance of auxin. The crux of it all is the relationship between auxin and self-weight. This competition is a robust relationship that leads to a much more accurate deflection of plant stems under gravitropic conditions.

Chapter Eight

Conclusions and Implications

8.1 Summary

Plant mechanics have intrigued numerous scientists and engineers for years dating all the way back to the time of the brilliant mind of Charles Darwin. The allure of understanding and reproducing such interactions with the surrounding environment has always been thrilling. Plants are biological entities that have a lifespan just like every human being. That fact alone is what makes the search for the precise mechanisms behind plant tropisms so enthralling. The potential and possible application is extraordinary and could change the world as we know it. Phototropism and gravitropism involve a plants reaction to light and gravity, respectively. These two environmental stimuli are omnipresent and comprehending how a plant incorporates them into its basic life cycle is vital.

The molecular level is the location in which insight to plant response for tropisms begins. In the case of phototropism, light is perceived by protein pigments called phototropins. They are responsible for detecting light and initiating a signal that is sent throughout the plant. PHOT1 and PHOT2 are distinct light receptors that localize to the plasma membrane of the plant cells. The localization brings about the activation of the cells through phosphorylation and proteins within the cell are activated. The PIN family of proteins has been found to activate and localize at the plasma membrane as well. These proteins then interact with the most important plant hormone within the system. The naturally occurring auxin registers the signal initiated by the activation of the plant cell proteins and moves laterally across the different cell layers of the stem.

As stated by the Cholodny-Went hypothesis, it is the gradient of lateral auxin movement that induces curvature.

Similar to phototropism, gravitropism involves a distinct portion of the plant registering the gravity vector. The endodermis layer of the plant shoot recognizes the force of gravity by the sedimentation of starch filled amyloplasts. They have the ability to sediment due to the fact that starch has a higher density than water and cells contain large sacs of water. As the plant is moving and growing against the vector of gravity, amyloplasts reorient themselves in the vacuoles of the cell to whichever side of the cell is considered the base. Since the vacuoles are a part of the cytoskeleton, the amyloplasts interact with the actin network that compiles the cytoskeleton. Tension within the network is modified and the cell is activated at the plasma membrane. Activation of the plasma membrane induces the ion channels that transfer the signal from the reorienting amyloplasts to the entire cell. From here, proteins are activated and localization at the plasma membrane occurs. Just like phototropism, the lateral movement of auxin is initiated and curvature then takes place.

Internal turgor pressure and cell wall compliance are the two main factors that are altered by auxin movement and provide cells with the ability to expand. Cell expansion is the distinct process behind curvature and is the key component to formulating a model. The processes of phototropism and gravitropism were exhaustively examined and an elementary model was assembled. The use of stress-strain relationships and curvature combined with the full comprehension of each tropism resulted in a model that effectively integrated turgor pressure and compliance into the system. A macro-micro approach was taken to establish equations for cellular response that could be applied over the entire plant shoot. The model was then put to the

test with benchmark problems and adequately passed each one. The only issue was the magnitude of the response.

A combination of testing values in Table 7.1 and the aid of personal plant testing added another facet to checking the validity of the model. Examination of research data rendered curvature responses that were similar to plants in their own environment. Several plots from Figures 7.3 to 7.7 depict the enhancement of the model and gravitropic curvature. Throughout each figure, the important stipulations found through numerous research articles were maintained. As auxin increases, the level of curvature should increase and the model displays that in Figure 7.3. With the increase of compliance and turgor pressure, the curvature response of the plant stem should increase as well. Figures 7.7 and 7.9 illustrate the conservation of such relationships. Gravitropic experimentation was utilized since reducing every variable except gravity was possible. After rigorous analysis, it was determined that various values needed changing because there was still a noticeable difference between the experimental data and the model. Overall curvature response improved significantly however something was missing.

The addition of the effect of the self-weight was the missing link to correct the model. From a modeling perspective, the deflected stem shape was taken as the new configuration and gravity loads were applied. Moment and curvature were calculated in the model which then allowed for the integration of curvature to find slope. This new slope was added to the original slope due to gravitropism. Integration of this new slope led to the total deflected shape due to gravitropism and self-weight of the stem. Figure 7.12 shows the addition of self-weight and the difference between the experimental data. After value manipulation, Figure 7.15 illustrates the model curvature as a close match to the experimental data.

Self-weight of the shoot is vital as well as its competition between auxin. Auxin has more of an effect on the model than self-weight but both factors play a large role in dictating the orientation of the shoot. In Figure 7.13, the change in auxin is much more powerful than the change of self-weight depicted in Figure 7.14. Nonetheless, the correlation between auxin concentration and self-weight is crucial. If the weight of the shoot is too great then the movement of auxin will not be enough to offset such large deflections. If the concentration of auxin is too high, then very large weights that are not in the realm of plant structures will be needed to reduce curvature. The perfect combination of self-weight and auxin concentration is found in Figure 7.15. Differences between the model and experimental data are small proving that the model has the ability to produce in situ curvature response of plant shoots.

8.2 Future Model Enhancements

The difference between this model and models used to replicate cell expansion and curvature is the factor of time. Another difference is the incorporation of auxin and how it is regulated with each tropism. In one research article, the mechanics of the cell wall and biomechanical responses to stresses are the main focus [62]. Differential equations are formed with time being the main variable that each function is integrated over. Time is an important factor that needs consideration if this model is to be further implemented. Through personal testing, both phototropism and gravitropism took time to visually observe and measure any significant values. In the case of gravitropism, the best deflection of a horizontally oriented stem occurred after a 4 hour interval of picture taking. Those cellular and molecular interactions within the plant stem take time and it is another factor that will only enhance the overall strength of this model.

However, where this model lacks with respect to time is gained through the derivation of how phototropism and gravitropism affect overall curvature. After rigorous data searching, one article was found to take into consideration phototropism and how it affects the Lockhart equation. The Lockhart equation is a time dependent function that examines the dynamic balance between the cells ability to intake water and cell wall compliance [63]. Essentially, it is an equation that looks at turgor pressure and the relaxation of the cell walls during the process of cell expansion. The article goes on to improve the Lockhart equation with the addition of phototropic response. The model composed in this paper used basic engineering and beam theory relationships to incorporate auxin and its role in tropic curvature. Results show that the model adequately produces the proper response with each tropism but improvements can be made. Perhaps the combination of the Lockhart equation and this current model can provide a stronger correlation between plant curvature and the plant hormone auxin.

8.3 Potential Applications

The purpose of modeling phototropism and gravitropism is to gather information and use it for a greater engineering application. Plants have the distinct ability to harness their surroundings and utilize that energy to maintain equilibrium. These biological structures are self-maintaining and manage to survive under the most extreme conditions. Biofabrication is a field gaining momentum in which biologically derived materials and biocatalysts are harnessed in the production of various structural elements [64]. Biological construction materials are being fabricated for the use of both process aids and structural materials [64]. There are still discrepancies with the inclusion of biological material but once those have been settled, a new form of nano-manufacturing may arise.

According to Armstrong and Spiller [65], synthetic biology has the potential to produce truly sustainable approaches for various structures. An opportunity may arise in which biological material is integrated into construction materials and self-sustaining edifices are erected. Synthetic biology combines the advantages of living systems with the robustness of traditional materials to produce genuinely sustainable and environmentally responsive architecture [65]. The possibility of a building utilizing the light given off from the sun to improve foundation elements is quite a realization. Obviously, massive amounts of research and testing are essential to promote this concept. However, the idea is there and a time where buildings are improving themselves and the surrounding environment may be around the corner.

Notation List

A	the area light on the stem after readjustment
a^a	auxin concentration in plant stem
a_0^a	initial auxin concentration
A_0	initial area of stem that light is shining on
$a_{,Y}$	auxin as is laterally moves through the stem
ARF	auxin response factor
Aux	auxin
C	compliance of cell wall
C_0, $(\bar{C})$	initial compliance
CA	centroidal axis
co	cortex of plant
$C_{,Y}$	compliance affected by auxin
CZ	central zone of apex also known as initial zone
dx	small change in length of the stem
dX	projection of dx with respect to the x' axis
dY	projection of dx with respect to the y' axis
$\frac{du}{dx}$	first derivative of u, strain in axial direction
$\frac{dv}{dx}$	first derivative of v, slope
$\frac{d^2v}{dx^2}$	second derivative of v, moment
E.D.	experimental data used for the model
en	endodermis of plant
ep	epidermis of plant

g	gravity vector
G	curvature times auxin, simplification of the model
G1	gap phase 1 of cell cycle
G2	gap phase 2 of cell cycle
H^+	hydrogen proton
IAA	indole-acetic acid also known as auxin
L	length of stem
I_1	light intensity on the left side of the stem
I_2	light intensity on the right side of the stem
K^+	potassium proton
l	length of cell after bending
l_0	initial cell length
l_1	length of cell after elongation
l_w	length of lateral cell wall
LP	leaf primordia
M	mitotic phase of cell cycle
OC	organizing center
P	turgor pressure
$P_0, (\bar{P})$	initial turgor pressure
$P_{,Y}$	turgor pressure affected by auxin
PZ	peripheral zone
R	radius of cell
RZ	rib zone also known as transition zone
S	synthesis phase of cell cycle
S	shoot orientation after phototropic response
S_0	initial stem orientation
S_{Axial}	shoot undergoing axial strain

S_{Bend}	shoot undergoing bending strain
SC	stem Cell
S_{Tot}	shoot orientation after deflection
t	cell wall thickness
u	infinitesimal displacement in the x direction
v	vacuole of cell
x’	coordinate used for horizontal axis
y’	coordinate used for vertical axis
v	infinitesimal displacement in the y direction, overall deflection when integrated
α	linear relationship between compliance and auxin
β	linear relationship between turgor pressure and auxin
ε	strain
ε_{Axial}	axial strain
ε_{Bend}	bending strain
ε_{TOT}	the addition of axial and bending strain
λ	relationship between stress and force
κ	curvature
$\frac{d\varphi}{dx}$	first derivative of slope, moment
φ	angle between the vertical and centroid of shoot
φ_{GRAV}	the slope of the shoot under gravitropic response
φ_{PHOT}	the slope of the shoot under phototropic response
ρ	radius of curvature
σ	stress from Hooke’s Law
$\delta\theta$	the angle of rotation
ξ	linear relationship between lateral movement of auxin and light intensity

References

1. Farsad, M., Vernerey, F. J., and Park, H. S. (2010). “An Extended Finite Element/Level Set Method to Study Surface effects on the Mechanical Behavior and Properties of Nanomaterials. International Journal of Numerical Methods in Engineering, DOI: 10.1002/nme.2946.

2. Whippo, Craig W, and Roger P. Hangarter. "Phototropism: Bending Towards Enlightenment." *The Plant Cell*. 18.5 (2006): 1110-1119. Print.

3. Knight, Thomas A. "On the Direction of the Radicle and Germen During the Vegetation of Seeds." *Philosophical Transactions of the Royal Society of London*. 96 (1806): 99-108. Print.

4. Frank, Albert B. *Beiträge Zur Pflanzenphysiologie*. Leipzig: s.n., 1868. Print.

5. Darwin, Charles R. *The Power of Movement in Plants*. London, 1880. Print.
6. "Dictionary of Botany." *Dictionary of Botany*. N.p., n.d. Web. 18 May 2012. <http://botanydictionary.org/>.

7. Rothert, W. "Uber Heliotropismus." *Beitr. Biol. Pflanzen* 7 (1894): 1-211. Print.

8. Gilroy, Simon, and Patrick H. Masson. *Plant Tropisms*. Ames, Iowa: Blackwell Pub, 2008. Print.

9. Iino, M, K Shimazaki, and M Wada. *Light Sensing in Plants*. New York: Yamada Science Foundation and Springer-Verlag Tokyo, 2005. Print.

10. Blaauw, A.H. "Licht Und Wachstum III. Meded." *Landbouwhogeschool Wageningen* 15 (1919): 89-204. Print.

11. Cosgrove, Daniel J. "Kinetic Separation Of Phototropism From Blue-Light Inhibition Of Stem Elongation." *Photochemistry and Photobiology* 42.6 (1985): 745-51. Print.

12. Kogl, F., and G.J. Schuringa. "Uber Die Inaktivierung Von Auxina- Lacton Bei Verschiedenen Welenlagen Und Den Einfluss Von Carotinoiden Auf Die Licktreaktion." *Hoppe-Seyler’s Z. Physiol. Chem.* 280 (1944): 148-61. Print.

13. Dickison, William C. *Integrative Plant Anatomy*. San Diego: Harcourt/Academic Press, 2000. Print.

14. "BaileyBio.com." *Bailey Bio*. N.p., n.d. Web. 13 July 2012. <http://www.baileybio.com/plogger/?level=picture>.

15. "Types of Meristems." *The Learning Garden*. N.p., n.d. Web. 13 July 2012. <http://assoc.garden.org/onlinecourse/PartI15.htm>.

16. Aichinger, E., N. Kornet, T. Friedrich, and T. Laux. "Plant Stem Cell Niches." *Annual Review of Plant Biology* 63 (2012): 615-36. Web.

17. Quint, M, and W.M Gray. "Auxin Signaling." *Current Opinion in Plant Biology*. 9.5 (2006): 448-453. Print.

18. Woodward, A. W. "Auxin: Regulation, Action, and Interaction." *Annals of Botany* 95.5 (2005): 707-35. Print.

19. Went, F. W., and Kenneth Vivian Thimann. *Phytohormones,*. New York: Macmillan, 1937. Print.

20. Davies, Peter J. *Plant Hormones: Physiology, Biochemistry, and Molecular Biology*. Dordrecht: Kluwer Academic, 1995. Print.

21. Muday, GK, and A DeLong. "Polar Auxin Transport: Controlling Where and How Much." *Trends in Plant Science*. 6.11 (2001): 535-42. Print.

22. Perrot-Rechenmann, C. "Cellular Responses to Auxin: Division Versus Expansion." *Cold Spring Harbor Perspectives in Biology*. 2.5 (2010). Print.

23. Palme, K., and G. Galweiler. "PIN-pointing the Molecular Basis of Auxin Transport." *Curr. Opin. Plant Biol.* 2 (1999): 375-81. Print.

24. Rubery, P.H. "Phytotropins: Receptors and Endogenous Ligands." *Symp. Soc. Exp. Biol.* 44 (1990): 119-46. Print.

25. Del Pozo, J. C. "The Balance between Cell Division and Endoreplication Depends on E2FC-DPB, Transcription Factors Regulated by the Ubiquitin-SCFSKP2A Pathway in Arabidopsis." *The Plant Cell Online* 18.9 (2006): 2224-235. Print.

26. Cosgrove, Daniel J. "Growth of the Plant Cell Wall." *Nature Reviews Molecular Cell Biology* 6.11 (2005): 850-61. Print.

27. Cosgrove, D. "Loosening of Plant Cell Walls by Expansins." *Nature* 407.6802 (2000): 321-26. Print.

28. Philippar, K., N. Ivashikina, P. Ache, M. Christian, H. Luthen, K. Palme, and R. Hendrich. "Auxin Activates KAT1 and KAT2, Two Kþ-channel Genes Expressed in Seedlings of Arabidopsis Thaliana." *Plant Journal* 35 (2004): 815-27. Print.

29. Ruck, Annegret, Klaus Palme, Michael A. Venis, Richard M. Napier, and Hubert H. Felle. "Patch-clamp Analysis Establishes a Role for an Auxin Binding Protein in the Auxin Stimulation of Plasma Membrane Current in Zea Mays Protoplasts." *The Plant Journal* 4.1 (1993): 41-46. Print.

30. Steffens, Bianka, Christian Feckler, Klaus Palme, May Christian, Michael Böttger, and Hartwig Lüthen. "The Auxin Signal for Protoplast Swelling Is Perceived by Extracellular ABP1." *The Plant Journal* 27.6 (2001): 591-99. Print.

31. Lenci, F, F Ghetti, G Colombetti, D -P. Häder, and Pill-Soon Song. *Biophysics of Photoreceptors and Photomovements in Microorganisms*. New York: Plenum Press, 1991. Print.

32. Cho, H.-Y., T.-S. Tseng, E. Kaiserli, S. Sullivan, J. M. Christie, and W. R. Briggs. "Physiological Roles of the Light, Oxygen, or Voltage Domains of Phototropin 1 and Phototropin 2 in Arabidopsis." *Plant Physiology* 143.1 (2006): 517-29. Print.

33. Sakamoto, Koji, and Winslow R. Briggs. "Cellular and Subcellular Localization of Phototropin 1." *The Plant Cell*. 14.8 (2002): 1723-1735. Print.

34. Christie, J.M., M. Salomon, K. Nozue, M. Wada, and W.R. Briggs. "LOV (light, Oxygen , or Voltage) Domains of the Blue-light Photoreceptor Phototropin (nph1): Binding Sites for the Chromophore Flavin Mononucleotide." *Proc. Natl. Acad. Sci. (USA)* 96 (1999): 8779-783. Print.

35. Sancar, Aziz. "Structure and Function of DNA Photolyase and Cryptochrome Blue-Light Photoreceptors." *Chemical Reviews* 103.6 (2003): 2203-238. Print.

36. Sakai, T., T. Wada, K. Ishiguro, and K. Okada. "RPT2: A Signal Transducer of the Phototropic Response in Arabidopsis." *Plant Cell* 12 (2000): 225-36. Print.

37. Cashmore, Anthony R. "CryptochromesEnabling Plants and Animals to Determine Circadian Time." *Cell* 114.5 (2003): 537-43. Print.

38. Parks, BM, MH Cho, and EP Spalding. "Two Genetically Separable Phases of Growth Inhibition Induced by Blue Light in Arabidopsis Seedlings." *Plant Physiology* 118 (1998): 609-15. Print.

39. Schepens, I., P. Duek, and C. Fankhauser. "Phytochrome-mediated Light Signalling in."*Current Opinion in Plant Biology* 7.5 (2004): 564-69. Print.

40. Hangarter, R. P. "Gravity, Light and Plant Form." *Plant, Cell and Environment* 20.6 (1997): 796-800. Print..

41. Liscum, E., and W.R. Briggs. "Mutations of Arabidopsis in Potential Transduction and Response of the Phototropic Signaling Pathway." *Plant Physiology* 112 (1996): 291-96. Print.

42. Blakeslee, J., W. Peer, and A. Murphy. "Auxin Transport." *Current Opinion in Plant Biology* 8.5 (2005): 494-500. Print.

43. Harper, RM, EL Stowe-Evans, DR Luesse, H. Muto, K. Tatematsu, MK Watahiki, K. Yamamoto, and E. Liscum. "The NPH4 Locus Encodes the Auxin Response Factor ARF7, a Conditional Regulator of Differential Growth in Aerial Arabidopsis Tissue."*Plant Cell* 12 (2000): 757-770. Print.

44. Fukaki, H, J Wysocka-Diller, T Kato, H Fujisawa, PN Benfey, and M Tasaka. "Genetic Evidence That the Endodermis Is Essential for Shoot Gravitropism in Arabidopsis Thaliana." *The Plant Journal: for Cell and Molecular Biology*. 14.4 (1998): 425-30. Print.

45. Tasaka, M, T Kato, and H Fukaki. "The Endodermis and Shoot Gravitropism." *Trends in Plant Science*. 4.3 (1999): 103-7. Print.

46. Sack, F.D. "Plant Gravity Sensing." *Int. Rev. Cytol.* 127 (1991): 193-252. Print.

47. Sack, FD. "Plastids and Gravitropic Sensing." *Planta*. 203 (1997): 63-8. Print.

48. Yano, D., M. Sato, C. Saito, M. H. Sato, M. T. Morita, and M. Tasaka. "A SNARE Complex Containing SGR3/AtVAM3 and ZIG/VTI11 in Gravity-sensing Cells Is Important for Arabidopsis Shoot Gravitropism." *Proceedings of the National Academy of Sciences* 100.14 (2003): 8589-594. Print.

49. Palmieri, M., and John Z. Kiss. "Disruption of the F-actin Cytoskeleton Limits Statolith Movement in Arabidopsis Hypocotyls." *Journal of Experimental Botany* 56.419 (2005): 2539-550. Print

50. Blancaflor, Elison B, and Patrick H. Masson. "Plant Gravitropism. Unraveling the Ups and Downs of a Complex Process." *Plant Physiology*. 133.4 (2003): 1677-1690. Print.

51. Friml, J, J Wiśniewska, E Benková, K Mendgen, and K Palme. "Lateral Relocation of Auxin Efflux Regulator Pin3 Mediates Tropism in Arabidopsis." *Nature*. 415.6873 (2002): 806-9. Print.

52. Taiz, Lincoln, and Eduardo Zeiger. *Plant Physiology*. Sunderland, MA: Sinauer Associates, 2010. Print.

53. Philpot, Timothy A. *Mechanics of Materials: An Integrated Learning System*. Hoboken, NJ: J. Wiley, 2011. Print.

54. Wilson, Reginald H., Andrew C. Smith, Marta Kacurakova, Paul K. Saunders, Nikolaus Wellner, and Keith W. Waldron. "The Mechanical Properties and Molecular Dynamics of Plant Cell Wall Polysaccharides Studied by Fourier-Transform Infrared Spectroscopy." *Plant Physiology* 124.September (2000): 397-405. Print.

55. Lodish, Harvey F. *Molecular Cell Biology*. New York: W.H. Freeman, 2000. Print.

56. Cosgrove, D. J. "Cell Wall Yield Properties of Growing Tissue : Evaluation by in Vivo Stress Relaxation." *Plant Physiology* 78.2 (1985): 347-56. Print.

57. Ryden, P., K. Sugimoto-Shirasu, A. C. Smith, K. Findlay, W.D. Reiter, and M. C. McCann. "Tensile Properties of Arabidopsis Cell Walls Depend on Both a Xyloglucan Cross-Linked Microfibrillar Network and Rhamnogalacturonan II-Borate Complexes." *Plant Physiology* 132.2 (2003): 1033-040. Print.

58. Shinkle, J. R., and W. R. Briggs. "Auxin Concentration/Growth Relationship for Avena Coleoptile Sections from Seedlings Grown in Complete Darkness." *Plant Physiology*74.2 (1984): 335-39. Print.

59. De Diego, N., F. Perez-Alfocea, E. Cantero, M. Lacuesta, and P. Moncalean. "Physiological Response to Drought in Radiata Pine: Phytohormone Implication at Leaf Level." *Tree Physiology* 32 (2012): 435-49. Print.

60. Kitamura, Sayaka, Akiko Mizuno, and Kiyoshi Katou. "IAA-Dependent Adjustment of the in Vivo Wall-Yielding Properties of Hypocotyl Segments of Vigna Unguiculata during Adaptive Growth Recovery from Osmotic Stress." *Plant Cell Physiology* 39.6 (1998): 627-31. Print.

61. Pedmale, U. V., and E. Liscum. "Regulation of Phototropic Signaling in Arabidopsis via Phosphorylation State Changes in the Phototropin 1-interacting Protein NPH3."*Journal of Biological Chemistry* 282.27 (2007): 19992-20001. Print.

62. Geitmann, Anja, and Joseph K.E. Ortega. "Mechanics and Modeling of Plant Cell Growth."*Trends in Plant Science* 14.9 (2009): 467-78. Print.

63. Pietruszka, M., and S. Lewicka. "Anisotropic Plant Growth Due to Phototropism." *Journal of Mathematical Biology* 54.1 (2006): 45-55. Print.

64. Wu, Li-Qun, and Gregory F. Payne. "Biofabrication: Using Biological Materials and Biocatalysts to Construct Nanostructured Assemblies." *Trends in Biotechnology*22.11 (2004): 593-99. Print.

65. Armstrong, Rachel, and Neil Spiller. "Synthetic Biology: Living Quarters." *Nature* 467.7318 (2010): 916-18. Print.

66. Jaillais, Yvon, and Joanne Chory. "Unraveling the Paradoxes of Plant Hormone Signaling Integration." *Nature Structural & Molecular Biology* 17.6 (2010): 642-45. Print.

67. Rakusová, Hana, Javier Gallego-Bartolomé, Marleen Vanstraelen, Hélène S. Robert, David Alabadí, Miguel A. Blázquez, Eva Benková, and Jiří Friml. "Polarization of PIN3-dependent Auxin Transport for Hypocotyl Gravitropic Response in Arabidopsis Thaliana." *The Plant Journal* 67.5 (2011): 817-26. Print.

68. Krogh, David. *A Brief Guide to Biology*. Upper Saddle River, NJ: Pearson Prentice Hall, 2007. Print.

69. Robson, Paul, Garry Whitelam, and Harry Smith. "Selected Components of the Shade-Avoidance Syndrome Are Displayed in a Normal Manner in Mutants of Arabidopsis Thaliana and Brassica Rapa Deficient in Phytochrome B." *Plant Physiology* 102 (1993): 1179-184. Print.

70. U.S. Department of Health and Human Services. *Inside the Cell.*Washington, D.C.: U.S. Department of Health and Human Services., 1997. Print.

71. Minc, Nicolas, Arezki Boudaoud, and Fred Chang. "Mechanical Forces of Fission Yeast Growth." *Current Biology* 19.13 (2009): 1096-101. Print.

72. Paul, T., L. Rahman, M.Z. Raihan, and M.B. Banu. "Development of Protocol for In Vitro Seed Germination and Seedling Attributes of Two Corchorus Species." *The Journal of Experimental Biosciences* 2.2 (2011): 11-16. Print.