Second Edition

# Plant Propagation Concepts and Laboratory Exercises

Edited by **Caula A. Beyl and Robert N. Trigiano**

CRC Press
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# Contents

# Acknowledgments

Foremost, just as in the first edition (2008), we wish to recognize the extraordinary efforts and talents of all the contributing authors—their creativity, support, advice, understanding, and patience throughout the conception and long-delayed development of the second edition of this book was phenomenal—and the Institute of Agriculture at the University of Tennessee for granting us the time and the financial support necessary to complete the book. We also thank our colleagues and students who suggested changes to the chapters and the format; which have improved the content and presentation of materials in the book. We also extend very special thanks to Randy Brehm, senior editor and good friend at CRC Press, who worked tirelessly to see this project through to completion and never lost confidence in us and our contributors in finishing the task. Lastly, RNT sincerely thanks PKT, RLB, CGS, ANT, DJG, and BHO for their advice, insight, friendship, love, and constant support and encouragement—RNT could have never done it without you and you have all made his life much richer. CAB thanks CAC for his talent, JPS for the careful reading, and her family for their unflinching support.

# Editors

**Caula A. Beyl**, dean of the College of Agricultural Sciences and Natural Resources, received a bachelor's degree in biology with majors in botany and zoology from Florida Atlantic University, Boca Raton, Florida, in 1974, and a master's degree in horticulture from Purdue University in West Lafayette, Indiana, in 1977. In 1979, she obtained a PhD from Purdue University in the area of stress physiology. She began her academic career as a postdoctoral researcher at Alabama A&M University, Huntsville, Alabama, and after a year, she joined the faculty in horticulture in the Department of Natural Resources and Environmental Studies. She was promoted to associate professor in 1987 and professor in 1992. Beyl provided leadership as a director of the Center for Plant Science within the Department of Plant and Soil Science from 1988 to 2002. In more than 33 years as a researcher, teacher, and administrator, she has served as a principal investigator or co-investigator on 42 funded research projects in various areas of horticulture, stress physiology, and space biology. She has been a major advisor to 8 doctoral candidates and 28 master's students, half of whom were minority students. Her research and academic work has resulted in 40 refereed research publications; 16 book chapters; and 117 abstracts, presentations, or proceedings, 19 of which were on institutional research topics. Beyl is a member of the American Society for Horticultural Science and the honor society of Gamma Sigma Delta. She served as the editor for the *Plant Growth Regulation Quarterly* from 2001 to 2005 and the associate editor in the area of environmental stress physiology for the *Journal of the American Society for Horticultural Science* from 2000 to 2002. In 1995, she received the School of Agriculture Outstanding Researcher award at Alabama A&M University and, in 1998, the Abbott award for outstanding research paper from the Plant Growth Regulation Society. In 2005, she won the Alabama A&M University Researcher of the Month award. As an undergraduate and graduate educator, Beyl has taught 14 different courses including agricultural leadership and a graduate-level scientific writing course, and was honored for outstanding teaching with the Alabama A&M University Outstanding Teacher award in 1998 and the School of Agriculture Outstanding Teacher award in 1998 and 1991. In 2003, she was the recipient of the Distinguished Alumna award from the horticulture program, Purdue University.

In 2002, she assumed the role of director of the Office of Institutional Planning, Research, and Evaluation. As such, she revitalized the office and helped revise and write new strategic and effectiveness documents for the university, and was essential in guiding Alabama A&M University through its reaffirmation of accreditation process, including the development of a Quality Enhancement Plan. The planning documents that she helped develop were honored by the Southern Association of Institutional Researchers with the Outstanding Planning Document award in 2004. She has served as a consultant to Bethune Cookman College, Bennett College for Women, and Southern University at Baton Rouge for SACS, QEP, and learning outcome development. She also served as the Quality Enhancement Plan evaluator for SACS during its on-site visit to the University of Central Florida. She has presented on a variety of retention, institutional planning, learning outcomes, and QEP development topics to NACDRAO, SAIR, SEF, and SACS, among others. She served as an Alabama Baldrige quality award examiner in 2006. In 2007, she was appointed interim dean of graduate studies at Alabama A&M University. Since joining the University of Tennessee in June of 2007 as the dean of the College of Agricultural Sciences and Natural Resources, she has been active in implementing strategies to focus on retention, recruitment, and diversity issues as well as in integrating technological paradigms into the classroom to enhance learning of the millennial generation. In 2008, she received the Outstanding College of Agriculture Alumna award from Purdue University, and she was inducted into the Alumni Hall of Fame of Florida Atlantic University. In the same year, she was elected fellow in the American Society for Horticultural Science and, in 2009, was honored with their Outstanding Graduate Educator award. As the dean of the College of Agricultural Sciences and Natural Resources, her time is spent developing global engagement for the college, teaching, guiding the college through its strategic planning process, and writing.

**Robert N. Trigiano** received a bachelor's degree with an emphasis in biology and chemistry from Juniata College, Huntingdon, Pennsylvania, in 1975, and a master's degree in biology (mycology) from Pennsylvania State University in 1977. He was an associate research agronomist working with mushroom culture and plant pathology for Green Giant Co., Le Sueur, Minnesota, until 1979, and then a mushroom grower for Rol-Land Farms, Ltd., Blenheim, Ontario, Canada, in 1979 and 1980. He completed a PhD in botany and plant pathology (co-majors)

at North Carolina State University at Raleigh in 1983. After concluding postdoctoral work in the Plant and Soil Science Department at the University of Tennessee, he was appointed an assistant professor in the Department of Ornamental Horticulture and Landscape Design at the same university in 1987 and promoted to associate professor in 1991 and to professor in 1997. He served as interim head of the department from 1999 to 2001. He then joined the Department of Entomology and Plant Pathology at the University of Tennessee in 2002 and was interim head from 2012 to 2013.

Trigiano is a member of the American Phytopathological Society and the American Society for Horticultural Science (ASHS), and the honorary societies of Gamma Sigma Delta, Sigma Xi, and Phi Kappa Phi. He received the T. J. Whatley Distinguished Young Scientist award (Institute of Agriculture, University of Tennessee) and the Gamma Sigma Delta Research Individual and Team Award of Merit at the University of Tennessee. He is the recipient of the publication awards for the most outstanding educational and ornamental papers in ASHS and the Southern region ASHS L. M. Ware distinguished research award. In 2006, he was elected fellow of ASHS. Trigiano was awarded the B. Otto and Kathleen Wheeley Award of Excellence in Technology Transfer and the founder and manager of Creative Agricultural Technologies, LLC, by the University of Tennessee Research Foundation in 2007. He has been an editor for *Plant Disease*; ASHS journals; *Plant Cell, Tissue, and Organ Culture*; and *Plant Cell Reports* and is currently the co-editor-in-chief of *Critical Reviews in Plant Sciences and associate editor for Horticultural Research*. Additionally, he has co-edited ten books, all published by CRC Press (Boca Raton, FL), including *Plant Tissue Culture Concepts and Laboratory Exercises* (two editions: 1996 and 2000), *Plant Pathology Concepts and Laboratory Exercises* (two editions: 2004 and 2008), and *Plant Tissue Culture Development and Biotechnology* (2011).

Trigiano has been the recipient of research grants from the U.S. Department of Agriculture and Forest Service and the Horticultural Research Institute, and from private industries and foundations. He has published more than 200 research papers, book chapters, patents, and popular press articles. He teaches a graduate course in scientific writing, has presented numerous workshops on English scientific writing in Germany, the People's Republic of China, and Brazil, and founded Scientific Writing Experts, an educational business. His current research interests include molecular markers for breeding ornamental plants, population studies of pathogens and native plants, diseases of ornamental plants, somatic embryogenesis, and micropropagation of ornamental species.

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# Part I

## Introduction

# 1 Introduction to Plant Propagation

*Caula A. Beyl and Robert N. Trigiano*

**Concept Box 1.1**

- Early Greeks, Romans, and Chinese knew and used various techniques of plant propagation, including rooting cuttings, air layering, and grafting.
- Knowledge of plant growth hormones and the role of the endogenous growth regulator, auxin, in promoting the initiation of roots was a major milestone in plant propagation development.
- Various methods are used to propagate plants, but ultimately, the methodology used depends on whether a plant is desired to be genetically identical to the original.
- Sexual processes result in propagation by seed, particularly important to the vegetable, bedding plant, and nursery industries.
- Asexual methods include division; cuttage using various parts of the plant, including stems, leaves, and roots; budding; and grafting.
- There are many challenges to successful plant propagation, including lack of knowledge, recalcitrance associated with the phase change from juvenility to maturity, and various pathogens that thrive in the propagation environment.
- Future approaches to plant propagation may include genetic and physiological studies of rooting, the manipulation through tissue culture to stabilize the juvenile phenotype, adjusting conditions in which stock plants are held to optimize the rooting of the propagules taken from them, and the use of special properties of certain bacteria such as *Agrobacterium rhizogenes* to induce roots, in this case, hairy roots.

## INTRODUCTION

Man's dependence on plants as the most significant source of food depends predominantly on the ability not only to cultivate plants and utilize them, but also to propagate them. This knowledge of plant propagation, the multiplication or making of more plants, is both a fascinating art and an exciting science. Merely walking through the produce department of a grocery store and examining the array of fruits, vegetables, and flowers available should engender an appreciation of all the various techniques of plant propagation used. If the plants themselves could reveal how they were propagated, an impressive array of techniques would be described from the relatively simple methods of seed germination and grafting to the more cutting-edge techniques of tissue culture, which often includes molecular genetics.

## HISTORY OF PLANT PROPAGATION

The origins of plant propagation are hard to document, but it is reasonable to believe that plant propagation co-developed with agriculture approximately 10,000 years ago. The earliest propagation may have been an inadvertent sowing of seeds gathered during collection and harvesting activities, which evolved into deliberate agriculture. Descriptions of early horticulture in Egypt, Babylon, China, and other countries suggest that the culture of ornamental and food crops was fairly well understood and that they could be propagated easily. The Old Testament contains the following passage from Ezekiel 17:22: "…I myself will take a shoot from the very top of a cedar and plant it. I will break off a tender sprig from its topmost shoots and plant it on a high and lofty mountain…it will produce branches and bear fruit and become a splendid cedar…" This indicates that the concept of taking cuttings was well known at that time. Babylon and Assyria were known for terraced gardens and parks. Such deliberate and extensive cultivation of ornamentals required knowledge of how they could be propagated. The Greek philosopher Theophrastus (371–287 B.C.), a student of Aristotle, made observations on the suckering of olive, pear, and pomegranate.

**FIGURE 1.1** Detached scion grafting, part of a larger mosaic calendar featuring different agricultural activities throughout the year found in St. Roman-en-gal, Vienne, France, from the third century C.E. (Mudge, K. W., J. Janick., S. Scofield, and E. E. Goldschmidt: A history of grafting. *Hort Rev.* 2009. 35. 437–493. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

With respect to grafting, he even wrote in *De Causis Plantarum*, "It is also reasonable that grafts should best take hold when scion and stock have the same bark, for the change is smallest between trees of the same kind…" Roman writings contain references to budding and grafting, and Roman mosaics depict grafting (Figure 1.1). Cato, a Roman statesman in the second century, described cuttings or scions of apple grafted onto sturdy rootstocks. Graftage and topworking were illustrated in medieval treatises (Figure 1.2).

The Wardian case, an interesting invention by Dr. Nathaniel Ward in the early 1800s, enabled not only the germination of fern spores and orchids, but also the transportation of newly germinated and delicate plants across

**FIGURE 1.2** 16th-century plate depicting the steps to be used for topworking trees successfully.

**FIGURE 1.3** Modern replica of the traditional Wardian case commonly used for the propagation of ferns and orchids and the culture of plants needing protection.

long distances. This enabled the plants to survive and arrive at their destinations in good condition. Wardian cases not only became quite popular for growing orchids, but also were instrumental in helping establish the tea plantations of Assam and rubber trees in Ceylon. Today, replicas of Wardian cases are still used by garden enthusiasts for propagating and protecting sensitive plants (Figure 1.3).

Much of modern plant propagation is unchanged from practices in use before the 1900s. The following types of cuttings that we use today were all used at that time: softwood, semi-hardwood, hardwood, herbaceous, leaf, and root. Propagators were aware that many cuttings root at the nodes and were maximizing cutting production by using single-node (leaf-bud) cuttings. Growers were propagating plants using bulbs, rhizomes, and stolons, with techniques such as separation and division. Both layering and grafting were used commercially before the 1900s, and methods used then remain largely unchanged today.

Transpiration of cuttings was controlled using high-humidity environments, such as glass-covered rooting chambers, some of which had supplemental bottom heat provided by lamps or recirculating water systems. Disease (likely caused by *Botrytis* and other pathogens) was a problem in these rooting chambers. Mist (Figure 1.4a) and fog (Figure 1.4b) for propagation were not developed until the 20th century.

The major discoveries and advancements in plant propagation in the 20th century are the developments of intermittent mist and fog systems; the discovery of plant growth substances; sanitation and disease control; the knowledge of juvenility and chimeras; micropropagation; and the use of micropropagation techniques to escape pathogens, such as fungi, viruses, mycoplasms, and bacteria.

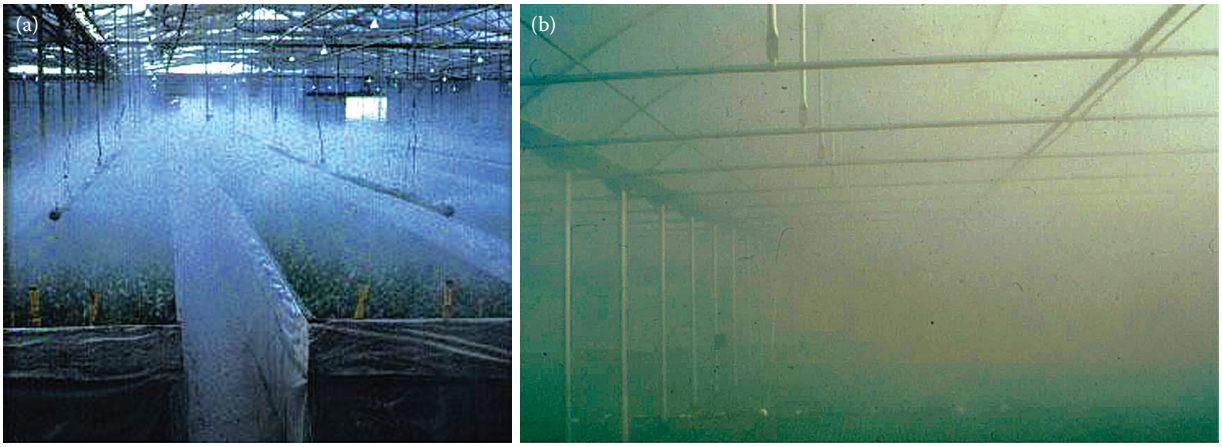


**FIGURE 1.4** Misting in greenhouse. (Courtesy of John Ruter, University of Georgia.)

## HEAT (STEAM) DISINFESTATION OF MEDIA

Many losses of propagated materials were incurred because of the presence of weeds and pathogens in the soil before the 20th century. In the early 1900s, growers were beginning to use steam to disinfest soil prior to planting. By the late 1930s to the early 1940s, people began disinfesting (pasteurizing) greenhouse and propagation media because the plants grew better than when the soils were sterilized by overheating. By the 1950s, people were aware that nitrifying and other beneficial microbes were killed by steam sterilization, and that has led to modern steaming methods aimed at killing insects, pathogens, and weed seeds while maintaining beneficial microbes and the physical structure of the media.

## DEVELOPMENT OF MIST AND FOG PROPAGATION SYSTEMS

Prior to the 20th century, transpiration from cuttings was reduced using high-humidity chambers; shading; cutting leaves in half and, otherwise, reducing leaf area; keeping the soil moist; and frequently sprinkling cuttings with water. By the 1940s and 1950s, intermittent mist and fog were being used for plant propagation. Commercial adoption of fog lagged behind mist because of disease problems. By the 1970s, ventilation combined with fog reduced the incidence of disease, and therefore, the use of fog in plant propagation has become more commonplace at commercial nurseries. Fog and intermittent mist are now indispensable in the cutting propagation of plants.

## AUXINS

One of the most important discoveries in the history of plant propagation were auxins and their role in root initiation. It was known for some time prior to the 1930s that the presence of leaves on cuttings was critical for adventitious rooting. In fact, it was known that concentrated "juice" from leaves could stimulate the rooting of cuttings. In the early 1930s, the auxin indole-3-acetic acid was isolated and shown to stimulate root formation. This hormone is produced in the growing points of shoots, especially in the young, expanding leaves. By the late 1930s, indole-3-butyric acid, naphthaleneacetic acid, and their commercial formulations "Auxan" and "Rootone" were available and being tested for rooting cuttings of many different species of plants. The use of auxin has increased the percentage of cuttings that root and the number and distribution of roots on cuttings. The quick commercial adoption of auxins for rooting speaks to the great need for root-inducing substances.

## MICROPROPAGATION

Early in the 20th century, pioneers in plant tissue culture first developed *in vitro* techniques for the germination of orchid seeds. Later, a medium was developed, which supported the growth of roots floating on its surface. However, *in vitro* culture was far from being commercially viable because of the lack of a plant growth regulator that would stimulate cell division. By the early 1950s, researchers had shown that water extracts from the vascular tissue of tobacco, malt extract, liquid *Cocos nucifera* L. (coconut) endosperm, and an extract from solid coconut endosperm all induced cell division. Additionally, autoclaved DNA from herring sperm and from calf thymus stimulated cell division in tobacco wound callus tissue cultured *in vitro*. This activity was not present if the DNA was not autoclaved.

Cytokinins are a class of growth regulators that induce cell (*cyto*) division (*kinin*). The first cytokinin, kinetin, was isolated, purified, and crystallized from autoclaved animal DNA and was shown to stimulate cell division and shoot multiplication. Since then, benzyladenine, thidiazuron, and many others have been

developed. These compounds are much more potent (effects expressed at much lower concentrations) cytokinins than kinetin and are in wide use in the commercial micropropagation industry today.

By manipulating the plant growth regulators, medium formulation, and plant materials, it is now possible to produce non-zygotic (somatic) embryos, adventitious shoots, axillary shoots, and adventitious roots using *in vitro* techniques. As a result, commercial micropropagation is an important part of the plant propagation industry.

## ESCAPING PATHOGENS

Plant diseases, especially those caused by viruses, are especially problematic because plants with viruses cannot be "cured." During the late 1940s, there was an outbreak of spotted wilt disease in *Dahlia pinnata* (Dahlia), which is caused by a virus. Tip cuttings rooted from infected plants showed no spotted wilt symptoms. By the early 1950s, scientists were excising very small 250-µm apical meristematic domes from dahlia plants with symptoms of dahlia mosaic virus and were placing them *in vitro* where they elongated. When the shoots were 1 to 2 cm long, they were grafted onto young virus-free plants, where they grew normally without dahlia mosaic symptoms.

By the mid- to late-1950s, scientists were combining thermotherapy with tissue culture. Virus-infected plants were grown at 25 to 40°C prior to excising meristematic domes and placing them *in vitro.* A percentage of the resulting plants were free from certain viruses by grafting onto indicator plants or using immunoassay techniques, such as enzyme-linked immunosorbent assay, which was developed in the 1970s for the detection of viruses. Therefore, plant pathologists have helped plant propagators in the 20th century by developing methods for steaming and disinfestation of media, benches, and planting containers. In turn, plant propagators have helped plant pathologists by developing techniques, including rooting of cuttings, grafting, and tissue culture, which can be used to escape viruses. Escaping viruses is an important part of the fruit, nut, and vegetable industries of the 21st century.

## ROLE AND IMPORTANCE OF PLANT PROPAGATION TODAY

Everything that is dependent on plants in any way is also dependent on the ability of plants to be propagated. Large-scale agriculture of crops, such as for grains and certain vegetables, depends on successful seed germination. Looking at it another way, you could say that seeds are the first necessary step in the world's food chain, and this dependency has resulted in a huge industry. The estimated value of the global domestic seed market was $45 billion per year for 2012, $12 billion of which was for the United States alone (International Seed Federation, 2013). The requirements to germinate seeds can be as simple as requiring appropriate moisture and temperature conditions or they can be as complex as requiring several months of moist, cold chilling treatment (stratification), or other specialized environmental conditions. Sometimes, the seed coat with its barrier to water penetration must be broken or weakened (scarification). Large numbers of seeds can be planted mechanically, but in some cases, they must be planted by hand to avoid damage and obtain a better stand such as for buckeye (*Aesculus parviflora* Walt.; Figure 1.5). Nursery industries all over the world use a variety of plant propagation techniques from T-budding dogwoods in Tennessee (Figure 1.6) and June-budding peaches in Georgia to stool-bedding apple rootstocks in England, a process taking a year or more. Nurserymen propagating ornamental trees, shrubs, and grasses or specializing in fruit trees must know the best way to propagate each species and have a thorough knowledge of the best "window of opportunity" that works for each species.

The floral and nursery industries are recognized as the two fastest growing segments of horticulture in recent years. The growth and demand for bedding plants has probably been the most significant factor in this growth over the last 20 years. Establishment of bedding plants is dependent on successful and uniform seed germination.

**FIGURE 1.5** Seeds of Ohio buckeye (*Aesculus parvifolia*) being planted by hand at Shadow Nursery, Winchester, Tennessee, to prevent damage and increase the number that will germinate successfully.

**FIGURE 1.6** Fall budding dogwoods in the field at Shadow Nursery, Winchester, Tennessee. The bud stick from which the bud of the desired cultivar was taken can be seen in the propagator's right hand. Notice that the budding rubber used to wrap the T-bud is wrapped from below the insertion point upward.

Another area of horticulture dependent on successful seed germination and production of transplants is the vegetable production industry. The Chinese described grafting of vegetable crops as early as the fifth century, and today, both Japan and Korea have developed robots and grafting machines to produce large numbers of grafted tomatoes, eggplants, and peppers.

Even the forest industry is very interested in plant propagation techniques being used to improve the performance of trees, from the establishment of seed orchards containing superior tree selections to the use of cuttings of superior clones. Some approaches have focused on non-zygotic embryogenesis or creating an embryo identical to the original from the somatic portion of the plant. Clonal approaches are being studied all over the world for species, such as eucalyptus and pine, and molecular genetics techniques are being explored to boost the genes for auxin production in aspen to increase how rapidly the transgenic tree will grow.

The craft and science of plant propagation is particularly important for the preservation of rare and endangered plants. Both traditional and specialized techniques are used to propagate species recognized as either rare or endangered so that they may be preserved and, in some cases, re-introduced into new and old habitats. Sometimes, these techniques involve *in vitro* seed germination, and in other cases, various tissues may be used in tissue culture to create a large number of plants very rapidly for species that respond well to the *in vitro* environment. Plant propagators working for arboreta and botanical gardens must have a thorough knowledge of plant propagation to be able to germinate seeds sent to them from all parts of the world or to increase numbers of rare or highly valued specimen plants to share with other arboreta and botanical gardens.

## HOW THIS BOOK IS ORGANIZED

This book is based on a successful model of organization used in *Plant Pathology Concepts and Laboratory Exercises*, Second Edition (Trigiano, Windham, and Windham, 2008), and *Plant Tissue Culture, Development, and Biotechnology* (Trigiano and Gray, 2010), which combines concept chapters with accompanying laboratory exercises to provide additional in-depth information and hands-on tasks that illustrate the principles found in the concept chapters. This not only aids those using the book for teaching, but also provides those using the book as a reference with examples of techniques that can be used as models for other species. The various chapters are contributed by horticultural scientists with expertise in the disciplines of plant propagation, breeding, pathology, tissue culture, seed technology, among others, and who have had extensive experience teaching plant propagation.

We have reorganized and revised this edition of the propagation book according to the many suggestions by our authors and instructors and, more importantly, students who have used the book. The most obvious change is that we have fashioned a beginning section that collects basic botanical information for those students who are unfamiliar with botany or just need a quick "refresher" on the subjects. Other chapters throughout the book still focus on traditional horticultural practices much like those found in the first edition. We have also added a few more laboratory exercises that we hope will better illustrate the concepts in the main chapters.

The concept chapters always begin with a list of some of the more important ideas, the concept box, contained in the section. These bulleted lists are intended to be a type of "executive summary" for the chapter as well as to alert students to the major points of the topics in the chapter. The laboratory exercises are meant to illustrate the ideas and procedures from the concept chapters. They are organized in a standard format throughout the book, including a brief introduction or introductory remarks to the topic. Teachers and students are provided a list of materials necessary to complete the experiments,

followed by step-by-step procedures that detail the exact methodology for that exercise. This book is unique in propagation teaching aids as it provides, in general terms and descriptions, what the anticipated results of the laboratory exercises should be. The last section of the laboratory exercises lists a set of questions that are intended to stimulate discussion and thought about the experiments. The laboratory exercises included in the book have been used repeatedly by our instructors and proven to be very reliable for classroom use. They provide a broad exposure of the concepts presented in the chapters to students of hands-on application.

We realize that instructors arrange topics for their propagation courses in a manner specific to meet the needs of their classes. The organization of the chapters in this book reflects that which we have been told is "typical" of many courses; however, most chapters have been written to be independent or a "free standing unit of information" regardless of their presentation position in the book. Therefore, this edition offers a collection of informational chapters and laboratory exercises that may be used in any sequence that provides a good fit for the course without loss of clarity and usefulness.

After the Introduction to the book in Part I, Chapter 1, Part II, deals with "Botanical Concepts for Plant Propagation," which should provide students with a solid foundation of basic plant science subdisciplines. Chapter 2 serves as a basic primer for anatomy and morphology, which is necessary to understand the cells, tissues, and organs of plants. We have chosen to retain the black-and-white photomicrograph format in this chapter for clarity. The next three chapters (3, 4, and 5) delve into the very interesting field of plant physiology, including a general chapter on basic plant physiology and how it impacts the propagation systems used (Chapter 3), followed by the very specific content area of plant growth regulators (Chapter 4). What horticultural propagation text would be complete without a chapter on sexual reproduction and breeding? Chapter 5 not only provides clear, easy-to-understand diagrams and explanations of mitosis and meiosis, but also addresses the processes of sexual reproduction in angiosperms and some genetic and breeding concepts. Once again, we have retained the black-and-white instead of colored photomicrographs in this chapter for clarity. Chapter 6 deals with juvenility. The change of phase of a plant from juvenile to physiologically mature impacts not only flowering and fruiting, but also propagation success. Cuttings of some species are very difficult to impossible to root once they have become mature, and propagators have used a number of different techniques to "restore" juvenility, such as serial propagation, severe heading back, and *in vitro* culture. This section ends with a chapter (7) on chimeras. Chimeras are plants composed of layers or sectors that have a different genotype, so depending on where new shoots arise to become new plants, they may have a different phenotype than the parent plant. The genotype is the heritable genetic blueprint for the plant, and the phenotype is the manifestation of the plant that results from putting that genetic blueprint to work—its structure, function, and behavior. The concept of chimeras is often difficult for students to understand, but our authors have written very clear, understandable explanations, and the chapter is beautifully illustrated.

In Part III, the book leads readers through a progression, beginning with propagation structures of different types and how these can be managed for more effective propagation in Chapter 8. Chapter 9 explores the rationale for the design of mist or fog propagation systems for the production of cuttings or for germinating seeds. Intermittent mist/fog systems are one of the most significant innovations in asexual propagation, enabling the successful propagation of many species by providing a high-humidity environment conducive to rooting. Chapter 9 compares the rooting performance of cuttings in a propagation tent with those rooted under mist. This chapter allows the study of the various control systems that determine misting frequency and how to determine whether the mist is being distributed uniformly across the bench. A complete book on plant propagation cannot neglect containers and media used for seed germination, cutting propagation, and acclimatization of cuttings. Chapter 10 details the types of media that take the place of soil and their characteristics since they provide many advantages over soil, including lightness and ease of handling as well as freedom from pathogens, pests, weed seed, or other contaminants. Chapter 10 also deals with the factors that must be considered before an appropriate medium can be chosen and gives procedures for determining bulk density and pore space, important characteristics of media.

Part IV, "Plant Propagation Diseases, Insects, and the Importance of Sanitation," contains five chapters. Sanitation can determine the success or failure of seed or cutting propagation and can make the difference whether a commercial propagation establishment is economically viable. Part IV deals with the major threats to successful plant propagation offered by plant diseases. In Chapter 11, the characteristics of various common pathogens are presented along with information on how they are typically spread. Chapter 12 includes an exercise that illustrates the impact of *Botrytis* and other propagation pathogens. It also contains laboratory exercises on the chemical control of gray mold, the effect of ventilation on its development, and the biological control of *Pythium*. These concepts are reinforced by laboratory exercises on how to disinfest soil and planting media in Chapter 13. Chapter 14 provides important information on the concept of producing specific pathogen-free plants and crop certification programs in Canada. Integrated

pest management, which has almost become ubiquitous in literature on greenhouse and nursery management, is detailed in Chapter 15, with a focus on its application to propagation systems.

Part V contains only one chapter, but it is a very important chapter for anyone who conducts plant propagation research, whether an undergraduate or a graduate student, or a professional in the discipline. Chapter 16 is concerned with how to evaluate propagation experiments, and collect, handle, and analyze data. This issue is ignored completely or mentioned only briefly in most books on plant propagation but is an important aspect of horticultural education.

Parts VI to XII explore the various types of propagation, starting with the use of stem cuttings in Part VI. In Part VI, there are four chapters: a concept presentation followed by three novel laboratory exercises that explore the general concepts for successful vegetative propagation. Chapter 17 introduces the concept of cloning plants using stem cuttings. Clones are exact copies of the original plant and are used extensively in horticulture. This chapter is followed by a laboratory exercise (Chapter 18) that explores the adventitious rooting of woody plants. We introduce in this edition of the book a chapter (19) illustrating some cutting-edge woody propagation techniques using long cuttings developed in Germany in the last decade. The last chapter in the section (20) investigates the rooting of cuttings from tropical plants.

In Part VII, propagation by leaf and root cuttings is explored. For many, the idea that you can make a new plant genetically identical to the first by taking a cutting, inserting it into a rooting medium, keeping it moist and humid, and then watching new roots form is tremendously exciting. Home gardeners share cuttings this way quite frequently. The types of cuttings and what kinds of conditions help them root successfully are explored in Chapter 21.

Many people are familiar with the use of stem cuttings, but leaves and roots can also serve as very effective propagules. Many plants can be successfully rooted from just the leaf blade, and others, from the leaf and the petiole (Chapter 22). Many home horticulturists do this with leaf and petiole cuttings of African violets rooted in water. For other plants, particularly those that sucker readily, roots can serve as a source of cuttings or can be forced to produce shoots that, in turn, can be used as cuttings. There are many examples among woody trees of new trees developing from the roots of older ones until dense strands are formed (aspen and sumac). Chapter 23 uses sumac, nandina, mahonia, and crape myrtle to demonstrate propagation by root cuttings.

Part VIII contains a single chapter (24) that describes the time-honored technique of layering to produce new clonal plants on their own roots. Layering involves techniques that encourage roots to form before the cutting is severed from the parent plant. Layering techniques probably were adopted through close observation of what occurs in nature with some shrubs or trees. Low-lying branches that come into prolonged contact with the ground may form roots at that point. The technique, although slow, is still valuable for some species that are extremely difficult to root with cutting propagation.

Techniques of grafting and budding in Part IX require skill as well as science. Depictions of early knowledge and application of grafting has occurred in historical depictions from the 16th century (Figure 1.5). Grafting and budding both involve combining two plants: one serving as the rootstock or bottom portion of the plant and the other serving as the top portion of the plant (Chapter 25). With grafting, the scion or a piece of stem containing several buds is cut so that it can be joined with the rootstock. With budding, the scion piece consists of only one bud. These techniques are extremely important in the propagation of many fruit tree species, including apple and peach. Chapter 26 offers a variety of very easy-to-complete laboratory exercises on grafting techniques using rose, coleus, and boxwood, and Chapter 27 demonstrates the art of grafting vegetables.

Part X deals with bulbs and plants with special structures. Chapter 28 describes the use of underground storage structures to propagate plants asexually. We take advantage of the underground storage organ, such as bulbs, corms, etc., and use various techniques to encourage the bulbs to form more propagules. The laboratory exercise in Chapter 29 includes experiments with the propagation of lily, a nontunicate bulb, and hyacinth, a tunicate bulb. Tunicate bulbs like onion are protected by a dry papery sheath—a modified leaf around continuous concentric layers. It also includes an experiment with Irish potato (a stem tuber) and dahlia (a tuberous root). The chapter ends with a general observational activity of many bulbous and tuber crops in the field.

In the next section (Part XI), the chapters describe the exciting world of micropropagation, where small pieces of tissue or even single cells can be grown in a sterile environment on media containing all the nutrients, minerals, vitamins, growth regulators, etc. that the tissue (or explant) needs to proliferate more cells (Chapter 30). The mass of cells that grows from that original explant is called "callus." Depending on what growth regulators are used, explants can be tweaked to form shoots and roots or, via another route, non-zygotic embryos. Because a sterile (axenic) environment is needed and explants have such precise requirements for what is contained in the tissue culture medium, micropropagation laboratories have certain requirements in common (Chapter 31). Many of the operations manipulating the explants and placing them on sterile autoclaved medium are conducted in sterile transfer hoods. In Chapter 32, the procedures for the micropropagation of mint are described from

disinfesting the shoots for use as explants and preparing the medium to placing them in the culture vessels. Chapter 33 describes the micropropagation of tropical plants, and Chapter 34 describes the special procedures used with woody plants.

The most fundamental classification of plant propagation systems is not by the technique used, but whether they are the result of sexual or asexual process. The previous parts of the book depicting the types of plant propagation have all dealt with asexual or clonal propagation, in which the propagule is an identical genetic copy of the original plant. Part XII of the book deals with seed propagation. Seeds, unless they are apomictic, are the result of the sexual reproduction of plants. Apomixis is a special case of seed propagation that is asexual. This occurs with some species of citrus and Kentucky bluegrass, among other species. The embryo results either from cells of the ovule or from an egg with 2n chromosomes, which can develop without fertilization. Details of sexual reproduction in plants are featured in Chapter 5, which includes examples of higher plants, ferns, and mosses. Understanding not only the processes involved in developing a seed, but also how to produce and germinate a seed are important. Chapter 5 also explores plant breeding, a process that is essential for the transfer of traits between cultivars and the development of new cultivars with enhanced traits. Chapter 35 deals with seed production, analysis, and processing, and provides a laboratory exercise to emphasize the topic. Seeds have the potential to germinate into seedlings, which then develop into plants. Some seeds only require appropriate temperature and moist conditions to germinate readily. Others require much more complex conditions before they will germinate. Scarification treatments are used to weaken or remove hard seed coats, which are impediments to germination. Some seeds also require a period of cold moist stratification. In Chapter 36, the laboratory exercises acquaint students with various techniques used for scarification and the effectiveness of scarification coupled with stratification. Finally, some seeds are allowed to imbibe or take up water, which allows the initial events in germination to occur, but they are prevented from germinating fully by imbibing them in a germination solution containing solutes or an osmoticum, which keeps germination from proceeding beyond imbibition. This is called "priming," and its effects are explored in one of the laboratory exercises. When the seeds are dried and then imbibed again, they germinate much faster for having had the head start. The last exercise in that chapter deals with the stimulation of germination by the chemical constituents of smoke. Chapter 37 contains information on producing seedlings and bedding plants, without which garden centers in spring would be much less colorful and gardens would not get an early start.

The last section (Part XIII) contains chapters that are best described as "in conclusion: special topics." Chapter 38 takes a light-hearted, often humorous, and sometimes hilarious look at some propagation myths—those "truths" handed down to you by your grandmother. This chapter may also be described as "myth busters." Intellectual property protection for plants (Chapter 39) has become an increasing important concern for the horticultural industry in the last several decades. Horticulture is a big business, and tremendous resources are invested in developing new plants. These investments are now typically protected by legal processes, which may include national and international jurisdictions. This chapter provides an overview of plant protection instruments, which may take the form of trademarks, copyrights, patents, and plant breeder's rights. It also outlines the process for the awarding of exclusive rights to plants and briefly addresses the remedies or the enforcement of these rights. Many of the policies and rules governing patents changed in 2013, so this is a MUST read. The last chapter (40) speculates about the future directions of plant propagation research and some of the most interesting recent discoveries impacting the discipline that have come from the study of molecular genetics.

Lastly, we have included a DVD along with the book, which contains most of the figures appearing in the book. The figures are presented in color, with captions and/or notes in PowerPoint format, which are amenable to classroom or individual study. We have intentionally "mounted" the figures on a blank background so that they may be easily incorporated into your own presentations. Supplemental photographs have been added to some of the chapters to further illustrate important concepts.

## FUTURE OF PLANT PROPAGATION

Many different approaches are being pursued to develop new methods of plant propagation, and these include a better understanding of the genetic and physiological bases of rooting; the manipulation through tissue culture to stabilize the juvenile phenotype; adjusting conditions in which stock plants are held to optimize the rooting of the propagules taken from them; and the use of special properties of certain bacteria such as *Agrobacterium rhizogenes* to induce roots, in this case, hairy roots. Another approach may employ genetic engineering techniques to identify genes that contribute to the ease of rooting and insert those genes into more recalcitrant species.

Just examining the scientific literature devoted to plant propagation and its various techniques such as tissue culture can be overwhelming. For someone wanting the latest information about plant propagation

and the physiology associated with the induction of rooting, a membership in the American Society for Horticultural Science (ASHS) would be an excellent approach. ASHS provides outlets for peer-reviewed research in its three journals, which range from predominantly applied research to somewhat basic research. Another excellent source of current information about the latest techniques and how they have been applied to various species that are difficult to propagate is the International Plant Propagator's Society. Since 1951, this society has been sharing information about plant propagation. It has eight regions, including three in the United States—the Eastern, Southern, and Western regions. Attending its meetings is a delight for anyone who wants to focus specifically on propagating plants.

As you learn more about plant propagation in the following chapters, you will appreciate the variety of techniques used to propagate plants and the diversity of the kinds of propagules used. We hope that this knowledge will prepare you to be successful in your future plant propagation endeavors. We look forward to receiving any comments or suggestions that you may have to improve either the content or the presentations in the book and the DVD.

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# Part II

## Botanical Basics

# 2 A Brief Introduction to Plant Anatomy and Morphology

*Robert N. Trigiano, Jennifer A. Franklin, and Dennis J. Gray*

**Concept Box 2.1**

- Plant cells can be classified into the following basic types: meristematic and their immediate derivatives—parenchyma, collenchyma, and sclerenchyma.
- Simple tissues consist of one cell type, whereas complex tissues contain more than one of the basic cell types.
- The four plant organs are roots, stems, leaves, and flowers.
- Generally, the organization of monocots and dicots are similar, but they each have distinctive arrangements of cells/tissues in their organs.
- The four units of a flower are sepals, petals, anthers, and pistils.
- The study of plant anatomy is useful for understanding plant development, and determining the origin of cells and tissues *in situ* and *in vitro*.

This chapter, "A Brief Introduction to Plant Anatomy and Morphology," originally appeared in *Plant Tissue Culture, Development, and Biotechnology* (Trigiano and Gray, 2010), but is very appropriate for beginning propagation students. We included this chapter because many horticultural students lack basic botanical knowledge about how plants are assembled. We have also elected to retain the figures as in the original chapter as structures are exceeding clear in black and white. This chapter, therefore, explores some of the internal organization (cells, tissues, and organs) or anatomy of vascular plants. For simplicity, we have organized and illustrated the material by first looking at cell types and then comparing and contrasting the anatomy of the tissues and the organs of the monocotyledonous (monocot) and dicotyledonous (dicot) angiosperms, gymnosperms, and pteridophytes (ferns).

For the purposes of this book, we will consider the following four plant organs: roots, stems, leaves, and flowers (reproductive structures). Note that some authors omit the flower as an organ. It is impossible to discuss adequately all the details of the anatomy and development of these organs in this short chapter. Therefore, most treatments of cell types, tissue, and organs are described in broad, widespread terms, and students are cautioned that many exceptions to our generalizations can be found. The relationship of anatomy to the common forms and shapes, or morphology, of these organs will also be touched upon. Readers with greater interests in more exhaustive details of anatomy and development are directed to some botany and anatomy textbooks cited at the end of this chapter. Most of the material in this chapter is derived from Esau (1960) and Fahn (1990). Readers should also appreciate that while plant anatomy and morphology typically are studied through the use of static materials, such as histological sections, it is important to view these in the context that they represent growing, changing, three-dimensional organisms. In this way, an understanding of plant development can be better achieved.

Plants, like other complex organisms, are constructed of cells, the basic unit of life. However, unlike animal cells, plant cells are surrounded by a wall composed of structural polymers, which may include pectin, cellulose, lignin, and hemicellulose. The wall may be relatively thin and flexible as in many parenchyma cells or rather thick as in collenchyma and sclerenchyma cell types (see later discussion). In fact, the structure of the plant cell wall imparts, to some degree, the function of the cell. Cells may only have a primary cell wall, which is more or less defined as the wall material deposited while the cell is

increasing in size and having cellulose microfibrils that are laid down randomly or in more or less parallel orientations (Esau, 1960). The primary wall usually contains cellulose, hemicellulose, and pectic compounds referred to as the "middle lamella." The wall is flexible and stretches as the cell grows, with the orientation of cellulose fibers determining the direction of growth and eventual cell shape. The middle lamella acts as a "cement" between adjacent cells. Secondary walls found in some cells are deposited to the inside of the primary wall and the middle lamella after the primary wall has been completed and can be very thick. Lignin may then be deposited into primary and secondary cell walls, making the cell rigid. Secondary walls containing cellulose and hemicellulose and lignin may or may not be present (Esau, 1960).

## CELL TYPES

Let us consider the basic cell types of plants before examining the internal arrangement of cells into tissues and, in turn, tissues into organs. For the purposes of this chapter, plant cells can be simply classified into the following types and their variations: (1) meristematic, (2) parenchyma, (3) collenchyma, and (4) sclerenchyma. Note that most references consider the meristematic cells to be a form of parenchyma cells.

1. Meristematic cells have the following characteristics:
   - Very thin-walled cells that undergo mitosis to increase the length (apical meristem) or thickness (lateral meristem) of the organ. The meristematic initials reproduce themselves as well as form new cells, termed "derivatives," which increase the body of the plant. These derivative cells usually continue to divide several times before any significant differentiation into other cell types occurs. The initials and their derivative cells constitute the apical meristem (Esau, 1960), which can be found at the shoot (Figure 2.4a) and root (Figure 2.1a, b) tips. The stem apical meristem of monocots and dicots may be divided further into the tunica and the corpus. The tunica (coat) is one to several cell layers thick, divides only by anticlinal divisions to increase the surface area of the tip, and surrounds the corpus (body), which consists of a number of cells that divide in different planes to increase the volume of the meristem. This two-part arrangement of the meristem is absent in roots. The apical meristem of ferns and most other seedless nonvascular plants consists of a single large, triangular cell from which the surrounding apical meristem cells are derived, and the tunica/corpus organization is not present. In gymnosperms, there is no clear outer layer of tunica, but zones can be discerned within the apical meristem. An upper, lens-shaped region of large cells called the "central mother cell zone" and the surrounding

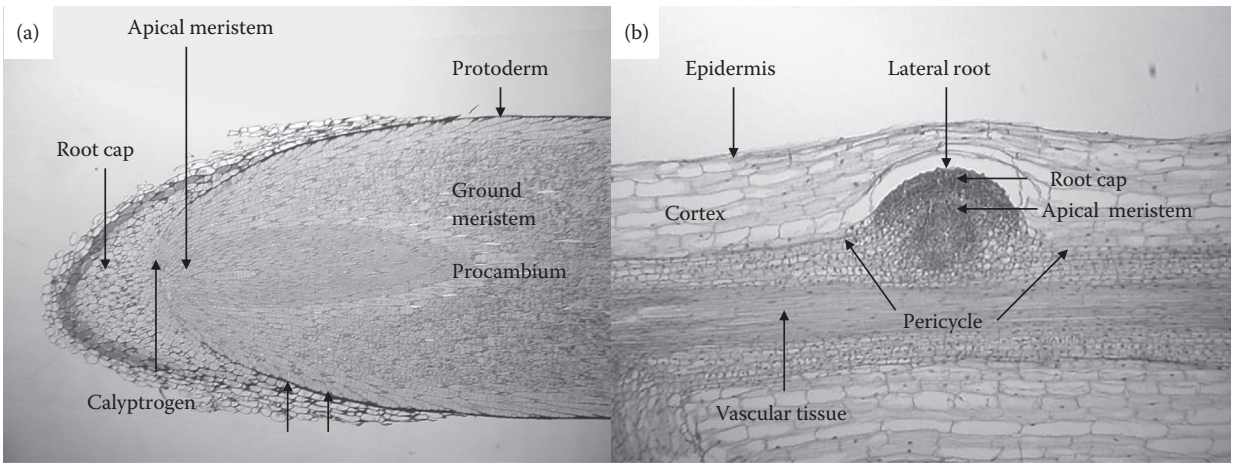


**FIGURE 2.1** Root apical meristem and lateral root origin. (a) Near-median longitudinal section through a young corn (*Zea mays*) root. The calyptrogen, in this case, gives rise to the cells of the root cap. In other roots, including those of many dicots, the root cap is derived from the apical meristem. The three meristematic areas are the protoderm (gives rise to the epidermis), the ground meristem (cortex), and the procambium (vascular tissue). Arrows indicate mucilaginous wall substance. Slide courtesy of Carolina Biological Supply Co., Burlington, North Carolina. (b) A young lateral (secondary) bean (*Phaseolus vulgaris*) root that originated from the pericycle. Note that, as the lateral root develops, it pushes through and crushes the cortical and epidermal tissues of the primary root. The architecture of lateral roots is similar to that of primary roots.


**FIGURE 2.2** (a) Roots of *Pinus* colonized by light-colored *Scleroderma* mycorrhizal hyphae, showing a cluster of the short, highly branched roots characteristic of many ectomycorrhizal associations. (b) Roots of *Castanea dentata* colonized at their tips by the dark hyphae of the ectomycorrhizae *Cenococcum*. (Photographs courtesy of Jenise Bauman.)

initials derived from it divide infrequently. Cells on the periphery of this zone are active in cell division, and below the apex, the arrangement is similar to that of monocots and dicots. The shoot apical meristem can be divided into a peripheral zone, which gives rise to leaves, buds, and flowers (lateral organs), and a rib zone, which produces the stem tissues. Several cell layers distal to the apical meristem and in the rib zone, the procambium (vascular), ground meristem (cortex), and protoderm (epidermis) of the stem are differentiated and give rise to the primary tissues of the plant body.

- Primary growth of the plant is brought about by the activities of apical meristems and subsequent divisions and the differentiation of the derivative cells into the tissues and the organs of the plant. All tissues originating from a primary meristem are termed "primary tissues," that is, the primary xylem and the epidermis. Most ferns, monocots, and some dicots complete their growth and development via primary growth only.
- Secondary growth exhibited by many dicots and gymnosperms is achieved through specialized lateral meristems. The vascular cambium (Figures 2.3b and 2.5a), located between the primary phloem and the primary xylem, produces cells that differentiate into additional vascular tissue, that is, the secondary xylem and the phloem, which increases the girth of stems and roots. Another lateral meristem, the phellogen or cork cambium, is found near the exterior of stems and roots and arises in the primary cortex. It produces phellem and phelloderm cells that replace the epidermis and the cortex, respectively, which is lost or crushed due to the expanding diameter of the root or the stem. Collectively, this new tissue is called the "periderm."

2. Parenchyma cells generally have the following characteristics:
   - They are typically nearly isodiametic (about as long as they are wide); however, cells may vary in shape, being elongated or even lobed.
   - The primary cell wall of this cell type is relatively thin and composed mainly of cellulose and hemicellulose, with a layer of pectic substances, the middle lamella, on the exterior of the primary wall. Note that some parenchyma cells, especially in vascular tissue, may develop a secondary wall or become sclerified with lignin (see sclerenchyma cells).
   - Parenchyma cells always have a nuclei and functioning protoplasts (cytoplasm).
   - These cells are generally considered to be relatively undifferentiated (compared to sclerenchyma) and capable of resuming meristematic activities by dedifferentiation. Indeed, this cell type and tissue is involved in the development of adventitious roots and shoots, wound healing, and other activities. Note that some parenchyma cells can be very differentiated and specialized in their function.
   - This cell type can be found through the body of the plant in primary and secondary tissues.

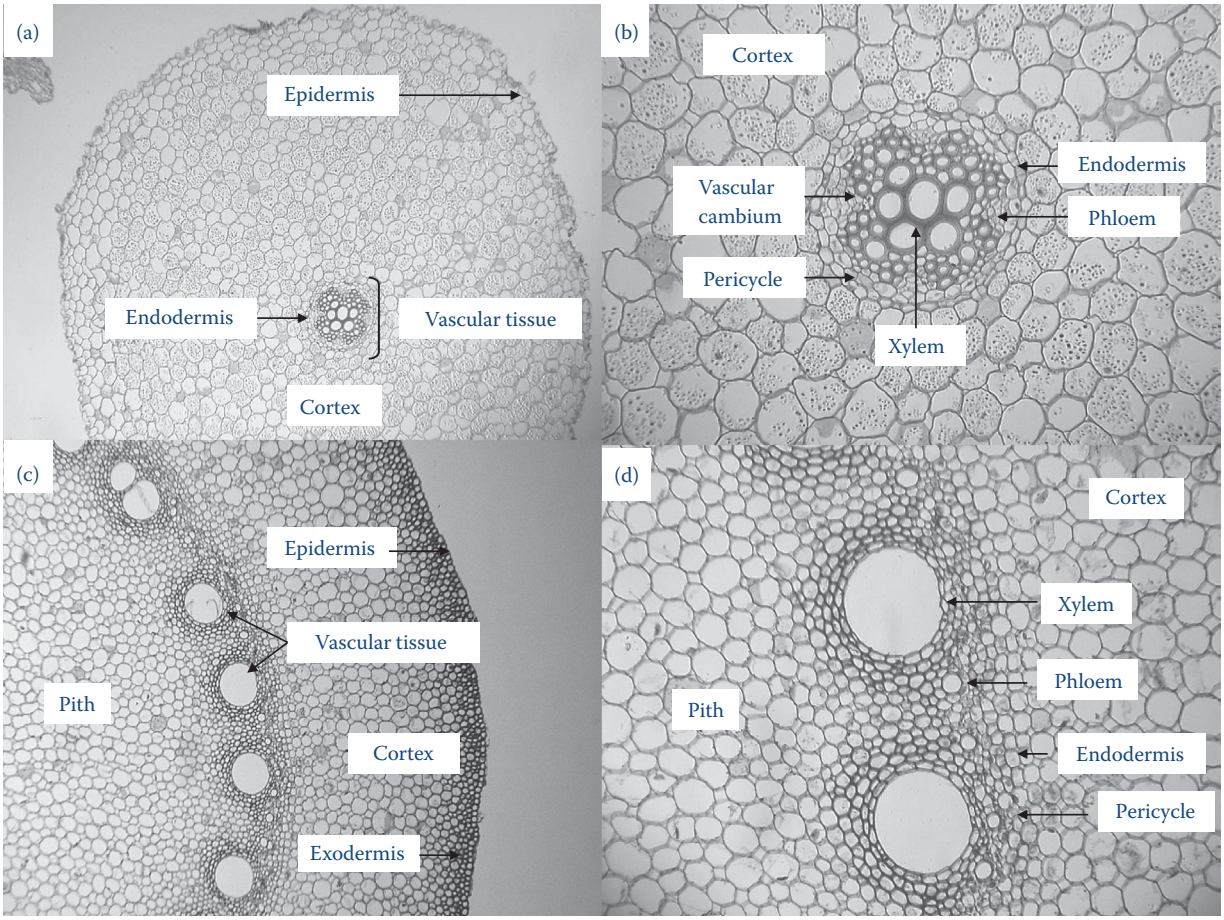


**FIGURE 2.3** Dicot and monocot root structure. (a) Cross section of a buttercup (*Ranunculus* species), a dicot root. Note the arrangement of tissues and the lack of pith in the center. (b) Higher magnification of the central area shown in Figure 2.3a. The "stele" includes all vascular tissue (xylem and phloem), the vascular cambium (discernible only by relative position in the young root), and the pericycle. It does not include the endodermis. Note the large xylem vessels in the center and the thickened walls (Casparian strips) of the endodermal cells. (c) Cross section of a corn (*Zea mays*), a monocot root. Note the central core of pith and very large xylem vessels. (d) Enlargement of the vascular area of the cross section shown in Figure 2.3c. (All slides provided courtesy of Carolina Biological Supply Co., Burlington, North Carolina.)

3. Collenchyma cells have the following characteristics:
   - They are typically more elongated than parenchyma cells and are specialized to function as a mechanical support for the plant.
   - Collenchyma cells have soft, pliable, unevenly thickened primary walls composed mostly of cellulose, with some pectin, but never lignin.
   - Collenchyma cells are similar to parenchyma cells in having a nuclei and a living protoplasm and are capable of dedifferentiation and meristematic activity.
   - This type of cell is generally found in young stems (Figure 2.5d) and leaf petioles and functions as flexible supporting tissues.
4. Sclerenchyma cells have the following characteristics:
   - Sclerenchyma cells can be long and thin (fibers; Figure 2.5a) or isodiametic to elongated (sclerids) and are involved in mechanical support and water conduction. This cell type is widely distributed in the four major plant organs. Specific cells types include fibers, vessel elements, tracheids, and sclerids (various forms including astrosclerids [star-shaped], stone cells, etc.).
   - The hallmark of sclerenchyma cells is the deposition of a secondary wall on the interior of the primary wall. Proceeding from the outside of the cell inward, the wall layers encountered in these cells are the middle lamella, the primary wall, and, lastly, the secondary wall. By definition, the secondary wall is laid down after the growth of the

cell ceases. The wall is composed primarily of cellulose arranged in parallel fibers and usually lacks pectic components. If a cell becomes lignified, deposition of lignin starts at the middle lamella and proceeds inward.

- Although many sclerenchyma cells are dead (lack a protoplasm) at functional maturity, some types of cells may retain a living protoplasm. However, the protoplasm of these cells appears to be physiologically nonfunctional or inactive (Esau, 1960).
- Sclerenchyma cells are highly differentiated and are usually considered not to be capable of dedifferentiation and resuming meristematic activity.

Cells are organized into simple tissues (one cell type) and complex tissues (more than one cell type). For example, young ground and pith tissue found in stems is a simple tissue made up of parenchyma cells (Esau, 1960), as is the mesophyll tissue in a leaf. Another example would be the collenchyma tissue found in the four corners of a mint stem (Figure 2.5d) or the "strings" in a celery stalk (petiole). An excellent example of a complex tissue is the secondary vascular tissues found in many dicots. This tissue contains representatives of both parenchyma and sclerenchyma cell types. Tissues, in turn, are organized into the four primary organs—roots, stems, leaves, and reproductive structures. The remainder of this chapter will be devoted to illustrating the general arrangement of cells and tissues within these organs and a brief account of seed anatomy. Anatomical studies of the tissue culture regeneration of organs are included where applicable.

## ROOTS

Roots serve as the primary water- and mineral-absorbing organ of plants. They also act to anchor the plant in the soil and may also function as storage organs and in vegetative (asexual) reproduction. Roots vary greatly in diameter, density of root hairs, and degree of lateral branching. Dicots and gymnosperms typically have a persistent taproot and may exhibit secondary growth, whereas with many monocots, the taproot is ephemeral and replaced with a fibrous root system consisting of many adventitious roots. The root morphology of ferns is fibrous and adventitious, similar to that of monocots, but the internal anatomy is similar with that of dicots.

While the general morphology of the root system is genetically determined, both overall appearance and internal anatomy can be modified by the growth environment. Primary roots typically range from 0.04 to 1 mm in diameter, with monocots often having roots that are smaller in diameter than those of dicots and gymnosperms. Very fine root-like structures produced by the fern gametophyte are greatly elongated single cells, rather than true roots, and are referred to as "rhizoids." Roots can contain a wide variety of pigments, and the color of roots ranges from white to brightly colored to nearly black. Young roots generally contain no pigments and, thus, appear white. Exposure to light may result in a pink pigmentation. However, in some species, the above-ground portions of roots contain chlorophyll, so are green in color. While older roots often appear to be darker in color, pigmentation is not an accurate indicator of maturity or internal anatomy.

The anatomy of roots is extremely variable, but general models may be developed for both monocots and dicots. The anatomy of ferns and gymnosperms is similar to that of dicots. The apical meristem of most roots (Figure 2.1a) appears less conspicuous than shoot meristems (Figure 2.4a), which are arranged as a tunica and a corpus. Ferns have a single large, triangular, meristematic apical cell rather than the multicellular meristem found in seed plants. In many plants, the root apical meristem gives rise to the cells of both the root cap and the primary meristems; however, in some grasses, a group of cells, the calyptrogen, produces the cells of the root cap (Figure 2.1a). In many instances, a quiescent ("quiet") zone is located in the apical meristem. This region exhibits low mitotic activity, but cell division typically resumes distal to this zone. The most noticeable differentiation of cells is in the vascular tissue—the primary phloem differentiates first, followed by the primary xylem. Cells continue to mature by elongation and enlargement. Root hairs, extensions of epidermal cells that increase the surface area to absorb water and minerals, are typically first seen behind the zone of mitotic activity and mark the maturation of the first xylem elements. Root hair length and density is genetically determined and fairly consistent within a species, but is highly variable between species.

Lateral or secondary roots greatly increase the absorptive area of the root system and are usually initiated from the pericycle, a layer or layers of cells in the vascular tissue or stele, at some distance behind the apical meristem (Figure 2.1b). Several adjacent pericycle cells divide to form a root primordium, and continued divisions force the developing root through and crush the endodermis, cortex, and epidermis of the primary root. Vascular tissue within the lateral root is connected to similar elements in the primary or parent root by differentiation of pericycle cells (Esau, 1960). The number of lateral branches is influenced by water, nutrient availability, and biotic interactions. Genetic and hormonal control of lateral branch production is fairly well understood in dicots, whereas less is known concerning the control of adventitious root proliferation in monocots (Osmont *et al.*, 2007). Associations with ectomycorrhizal fungi often result in clusters of short lateral roots that are covered with the fungal sheath. These are often visibly distinct, being dark in color, and occurring in clusters or in pairs

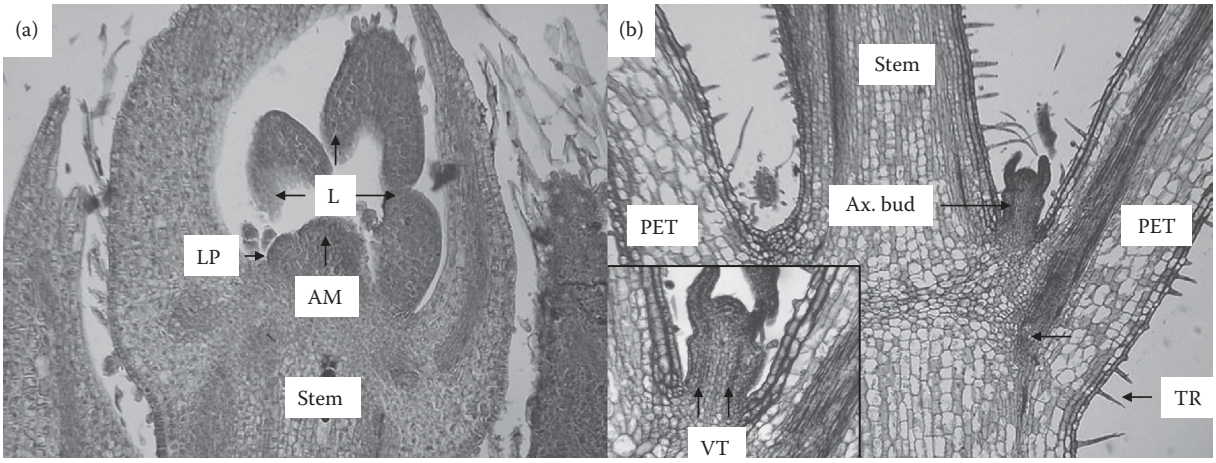


**FIGURE 2.4** Apical and lateral (axillary) shoot meristems. (a) Longitudinal section through the stem tip of bean (*Phaeseolus vulgaris*). The apical meristem (AM) is surrounded by a number of very young leaf primordia (LP) and more developed leaves (L). (b) Longitudinal section through a node of catnip (*Nepeta cataria*). The axillary bud (Ax. bud) is located in the axil formed by the leaf petiole (PET) and the stem. Note the vascular tissue (VT) extending from the petiole and connecting to the vascular tissue of the stem (arrow). The apical meristem (AM) is dome shaped, and the vascular tissue (VT) has differentiated (insert). TR = trichome.

(Figure 2.2a, b). The pattern of branching is termed "root architecture." Lateral roots growing directly from the first, or primary, root are termed "first-order lateral roots"; laterals growing from those are termed "second-order lateral roots," and so on.

Most of the same cells and tissues are present in monocots and dicots; however, the arrangement of these tissues is somewhat different. A summary of the main differences between the two divisions of angiosperms is presented in Table 2.1. The most conspicuous difference between the dicot and the monocot root anatomy is the arrangement of the primary vascular tissue. Most dicot roots lack central pith tissue, but instead, the core of the root is occupied by large metaxylem vessels with relatively large-diameter lumens (Figure 2.3a, b). Vascular tissue is contained in a single central area called the "stele," with the primary xylem arranged in arches or arms and the primary phloem located between the arms. Ferns generally have only two arms, termed "diarch development." Gymnosperms and dicots often have three to four arms, triarch or tetrarch development, although some species have as many as nine. A vascular cambium is located between the primary xylem and the phloem in dicot roots. In contrast, many primary monocot roots

**TABLE 2.1**

**A Summary Guide to the Morphological and Anatomical Traits of Monocots and Dicots[a]**

| Organ | Monocot | Dicot |
|---|---|---|
| Root | • Usually fibrous<br>• Pith present<br>• Lacks vascular and cork cambia or secondary growth | • Usually a taproot<br>• Lacks pith<br>• Vascular and cork cambia and secondary growth present<br>• Primary xylem typically arranged in "arches" |
| Stem | • Lacks pith, but vascular bundles embedded in pith-like fundamental tissue<br>• Vascular and cork cambia typically lacking; no secondary growth, but may have primary thickening meristems | • Pith present<br>• Vascular and cork cambia typically present; secondary growth evident manifested as rings of vascular tissue |
| Leaf | • Typically blade-like with parallel venation<br>• Leaf mesophyll generally undifferentiated into distinct layers | • Variously shaped with net venation<br>• Mesophyll may be differentiated into spongy and palisade parenchyma layers |
| Flower and seed | • Typically three-merous (flower parts in three or multiples of three)<br>• Embryo has one cotyledon | • Typically four or five-merous (flower parts in four or five or multiples of four or five)<br>• Embryo has two cotyledons |

[a] Although this table provides very broad characterizations and contrasts of monocot and dicot plants, the reader is cautioned that there are many exceptions to these generalizations.

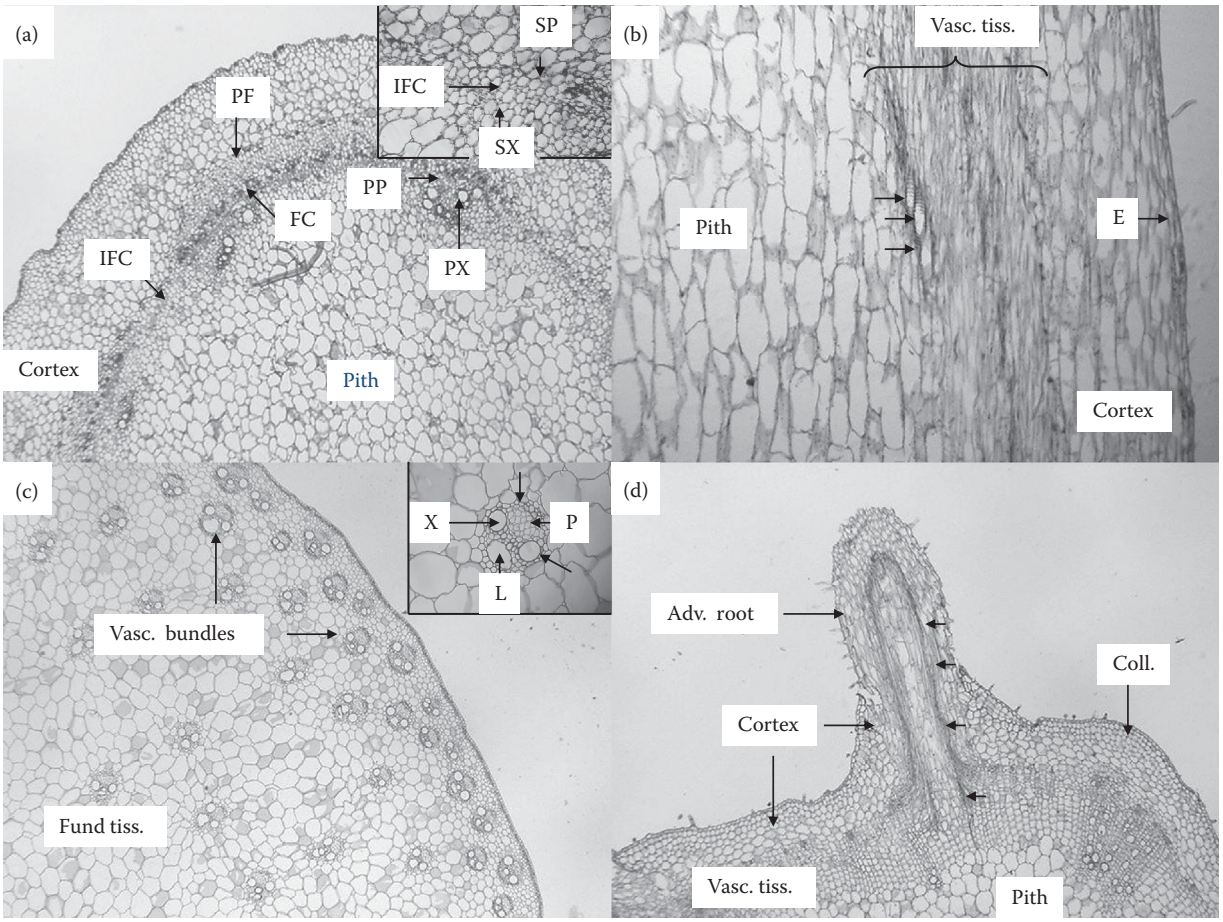


**FIGURE 2.5** Anatomy of dicot and monocot stems. (a) Cross section of a bean (*Phaeseolus vulgaris*) stem. Note the prominent pith and the cap of phloem fibers (PF). Secondary xylem (SX) and secondary phloem (SP) tissues, which originated from the interfascicular vascular cambium (IFC) and fascicular cambium (FC), are also common in dicot stems (insert). PP = primary phloem; PX = primary xylem. (b) Longitudinal section of bean illustrates the arrangement of tissues in the stem. Arrows indicate xylem vessels with helical secondary wall patterns. E = epidermis. (c) Cross section of an older corn (*Zea mays*) stem. Notice that vascular bundles (Vasc. bundles) are scattered throughout the stem and "embedded" in fundamental tissue (Fund. tiss.). The vascular bundles contain only primary xylem (X) and phloem (P) tissues (vascular cambium absent) with a prominent lacuna (L) or air space (insert). Arrows indicate sclerenchyma cells of the sheath. (Slide courtesy of Carolina Biological Supply Co., Burlington, North Carolina.) (d) Cross section through the stem of catnip (*Nepeta cataria*) and a longitudinal section through an adventitious root (Adv. root). The root originated near the primary phloem, and vascular tissue (arrows) has differentiated and connected to the vascular tissue (Vasc. tiss.) in the stem. Coll = collenchyma tissue.

have pith in the center of the primary root, surrounded by many arms of vascular tissue (Figure 2.3c, d). Dicot roots generally exhibit secondary growth via the vascular cambia located between the primary xylem and the phloem and by the cork cambia, which form in the cortex (see discussion in stems). Monocots and ferns lack lateral cambia or meristems.

Some species have roots that are highly specialized for a specific function, and these may have an internal structure that is somewhat different than the other portions of the root system. Dicots may have roots specialized for storage; these have a large diameter due to a proliferation of parenchyma cells within the secondary xylem and phloem, and may have several concentric layers of vascular cambia producing secondary vascular tissue. Aerial roots, or aerial portions of a root, generally lack root hairs, have smaller vessels than subterranean roots, and may be green due to the presence of chlorophyll in the cortex. Some roots, both above and below the ground, have a cortex that appears "spongy" to facilitate the diffusion of gases. Specialized sections of root may be produced to house associated organisms such as mycorrhizal fungi and certain types of bacteria. Specialized anatomy can also be found in parasitic roots.

## STEMS

As noted before, the shoot meristem (Figure 2.4a) of monocots and dicots is multicellular and organized into two parts—the tunica and the corpus—while that

of gymnosperms has a zonal organization. As in roots, the shoot apex of ferns is a single apical cell. Just as in the root apical meristem, the primary meristems—the ground, the procambium, and the protoderm—are also found in the shoot tip. However, the shoot tip is more complex than the root apex as it differentiates the leaves, the axillary buds (Figure 2.4a), and the flowers from the peripheral zone. The primary meristem at the end of each growing branch is the terminal bud. The stem of the plant is divided into nodes (leaves present) and internodes (leaves absent). There may be one, two, or several leaves at each node. Leaves are defined by the presence of an axillary bud (Figure 2.4b, insert) lying between the main stem and the leaf petiole (the axil). The anatomy of axillary buds is equivalent to the original shoot tip. The bud has the same structure as that of the apical meristem and serves to initiate branch or lateral growth and/or flowers under proper environmental conditions.

Stems are highly variable in form. The surface of the stem often has structures that reduce water loss and limit herbivory; trichomes and heavy cuticular waxes are present in many species. Trichomes may be a unicellular extension of the epidermis or made up of several cells. Scales are papery and one cell thick, and bristles and thorns are rigid, multicellular outgrowths of the epidermis. Many young stems and some older stems contain chlorophyll, so are green. Anthocyanins, which appear red or purple, are also common in stems. Green, photosynthesizing stems require a means of gas exchange, and this is provided by the stomata in very young stems.

The arrangement of tissues in stems is variable and very different than that in roots. Just as in root anatomy, dicot and monocot stems are generally different from one another, and these differences are summarized in Table 2.1. Dicots typically have primary vascular bundles (fasciculars) arranged in a ring around the central pith (Figure 2.5a, b). The primary xylem is located toward the pith, whereas the primary phloem is located toward and contiguous with the cortex. Note that, in some instances, the primary phloem may lie on either side of the primary xylem. This arrangement is common in ferns, which are similar in structure to dicots, although some primitive ferns lack pith. The fascicular may also have a cap of very conspicuous phloem fibers (Figure 2.5a). A portion of the vascular cambium (intrafascicular cambium) is located between the primary xylem and the phloem, and another portion (interfascicular cambium), between the vascular bundles. The activities of the vascular cambium produce secondary vascular tissues, which, in due course, surround the pith with a continuous ring of vascular tissue. The secondary vascular tissues eventually crush and obliterate the primary tissues, especially in perennials and woody plants. It is this continuous ring of vascular tissue that easily differentiates the pith from the cortex in dicots.

Dicots also have another lateral or secondary meristem—phellogen or cork cambium. This cambium or meristem is formed in the cortex or phloem and is responsible for forming additional cortical cells (phelloderm) and a replacement for the epidermis (periderm). Gas exchange is provided by lenticels, multicellular ruptures of the epidermis or periderm, in stems with secondary growth. These appear as dark or, more often, light-colored dots or patches on the stem.

In monocot stems, the vascular bundles are scattered throughout the ground or fundamental tissue, and as a result, a pith and cortex are not discernable (Figure 2.5c). The vascular bundles are typically surrounded by a sheath of sclerenchyma cells, which helps support the stem (Figure 2.5c, insert). Additionally, many monocot stems, for example, corn, have an abundance of small vascular bundles located just under the epidermis, imparting stiffness to the stem and helping the plant to withstand environmental stresses. Monocots lack vascular cambia and, therefore, do not have secondary growth. If stems thicken, they do so through the division of parenchyma cells in the ground tissue.

An important feature of stems, especially in the production of shoots in tissue culture or cuttings, is the ability to form adventitious roots (Figure 2.5d). These roots have their origin in parenchyma cells lying near the vascular (phloem) tissue, or from the interfascicular cambium, and grow similarly to the description provided for lateral roots. In many species, adventitious roots form most readily at nodes.

There are many modified stems among different plant taxa. Rhizomes are common in monocots and are the primary stem form of ferns. These grow horizontally underground, generally have short internodes, and produce both roots and leaves. Stolons are similar to rhizomes but are more often found in dicots, and generally have long internodes, grow horizontally above the ground, and produce a new individual at each node. Tubers, corms, and bulbs are below-ground stems modified for starch storage. The term "tuber" refers to a swollen below-ground structure. This can be a root, or a modified stem as in a potato, which can produce new shoots from axillary buds at, sometimes inconspicuous, internodes.

## LEAVES

Leaves are the primary photosynthesizing organs of vascular plants. Most angiosperm leaves are relatively thin and flat (large surface area compared to volume) and adapted for capturing light and facilitating gas/water exchange with the atmosphere. Fern leaves are similar, but are called "fronds." The leaves of most gymnosperms are elongated, may be round, oval, or triangular in cross section, and are commonly referred to as "needles." Other gymnosperms have small scalelike leaves that grow

closely oppressed to the stem. There are, of course, many exceptions to the above statements, and leaves can exhibit extensive, often unusual, modifications, depending on the environment coupled with genetic inputs and evolution.

Leaves are found at the nodes of the stem and are variously arranged (phyllotaxis)—alternate (one leaf at the node), opposite (two leaves at the node), or whorled (three or more leaves at the node). Some plants have leaves that are very difficult to recognize as leaves, whereas others are very obvious. Generally, leaves have three parts—blades or lamina, petioles, and stipules—although it is not unusual for many leaves to lack the petiole (sessile leaf) and/or the stipules. The blade may be simple with smooth or toothed margins; shallowly or deeply lobed; or compound, with the leaf divided into leaflets (Figure 2.6). There are many arrangements found in compound leaves, the variations of which are two common patterns: palmate, where the leaflets are all connected at the top of the petiole, and pinnate, with the leaflets forming two rows along the central midvein. All leaves must have an axillary bud associated with them, and the bud is located in the axis formed by the petiole and the stem. Compound leaves do not have buds at the base of the branch points or where leaflets join the common axis.

Many dicot and fern leaves exhibit net venation patterns with major and minor veins (Figure 2.6a), whereas monocots and gymnosperms typically have a parallel venation arrangement, with most veins of more or less equal size (Figure 2.6b). Note that there are many exceptions to this general rule. Many gymnosperms have a single vascular bundle, or two bundles that run side by side, down the length of the needle. Anatomically, the vascular tissue in the node will exhibit a leaf gap, parenchyma tissue in the vascular cylinder of the stem where the leaf trace(s) (vascular tissue connecting the leaf to the stem) is/are bent toward the leaf petiole (Esau, 1960).

Leaves typically do not exhibit secondary growth and, therefore, only have primary tissues. Leaf tissue can contain all the three basic cell types discussed previously. The epidermis of leaves has many special features, including a cuticle to prevent wall loss, various trichomes (hairs), guard cells and accessory cells to regulate water and gas exchange, and other cells with specialized functions (Figure 2.7a, b). Large bulliform cells in the epidermis of many grasses can "deflate," allowing the blade to fold or roll. A hypodermis beneath the epidermis of conifer needles protects inner tissue from mechanical injury. The leaf mesophyll of many dicots and ferns is differentiated into one or more palisade layers toward the adaxial (top) surface of the leaf, and the spongy mesophyll, toward the abaxial (bottom) surface. The palisade cells are elongated (shoe box–shaped) and arranged "tightly" and orthogonally (right angle) to the leaf surface. The cells of the spongy mesophyll tissue are isodiametric to slightly

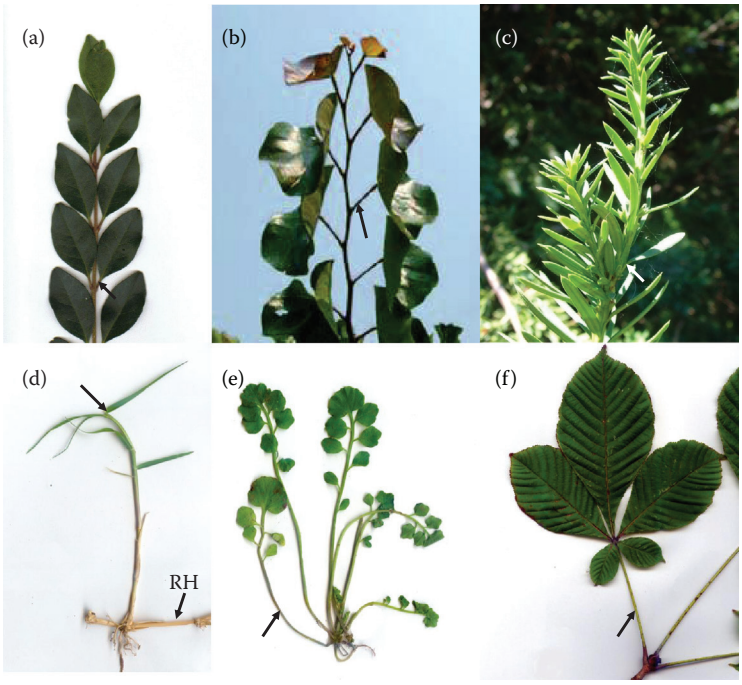


**FIGURE 2.6** Simple and compound leaves, with a petiole indicated by the arrow in each image. (a) Simple leaves with opposite leaf arrangement in privet (*Lingustrum japonicum*). (b) Simple leaves with an alternate leaf arrangement in redbud (*Cercis canadensis*). (c) Simple leaves of *Taxus* species, with whorled leaf arrangement. (d) Simple linear leaves of a monocot, a fescue grass (*Festuca* spp.). Note the horizontal stem, a rhizome (RH), from which the roots and the vertical stem are growing. (e) Pinnately compound leaves of *Cardamine hirsuta*. (f) Palmately compound leaf of buckeye (*Aesculus flava*).

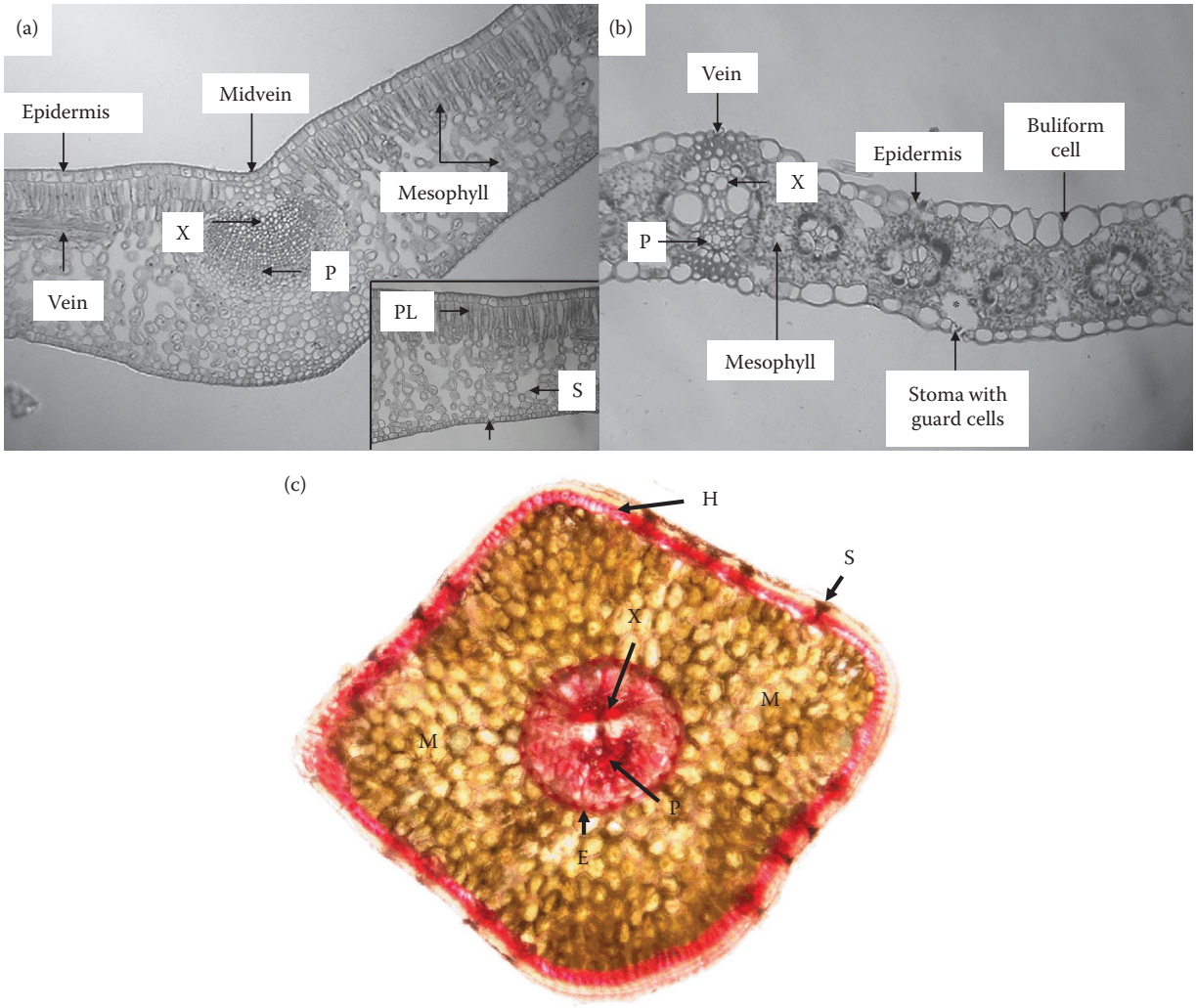


**FIGURE 2.7** Leaf anatomy. (a) Cross section of a dicot leaf (privet: *Ligustrum* species). A large midvein with xylem (X) toward the top (adaxial) surface and phloem (P) oriented toward the bottom (abaxial) surface is shown. The mesophyll is differentiated into palisade (PL) and spongy layers (S), with stomata and guard cells (arrow) on the bottom surface (insert). (b) Cross section through a corn (*Zea mays*) leaf, a typical monocot. Notice that the epidermis contains specialized bulliform cells and that the mesophyll tissue is not differentiated into layers. Stomata and guard cells are associated with large substomatal cavities (*). (a and b courtesy of Carolina Biological Supply Co., Burlington, North Carolina.) (c) Anatomy of a spruce leaf. Cross section of a gymnosperm leaf (*Picea pungens*). The single vein of xylem (X) and phloem (P) are encircled by the endodermis (E). The mesophyll (M) is dense and undifferentiated. Notice that immediately below the epidermis is a layer of sclerenchyma, the hypodermis (H), which contains channels for airflow from the stomatal opening (S) to the mesophyll.

elongated and arranged "loosely," with large intercellular spaces (Figure 2.7a). Xylem tissue is usually toward the top of the leaf, and the phloem, toward the bottom of the leaf. The mesophyll of monocot leaves and primitive ferns is typically not differentiated into layers and only has spongy parenchyma (Figure 2.7b). Vascular tissue exists as bundles running through the mesophyll of the leaf. The mesophyll of gymnosperms is also undifferentiated and compact, often having highly invaginated cell walls. Large, circular resin ducts may be found within the mesophyll. The vascular tissue is surrounded by transfusion tissue, made up of parenchyma and thin-walled tracheids, which, in turn, are surrounded by a suberized endodermis, similar with that of roots (Figure 2.7c). In many plants, the vascular tissue may be surrounded by a sheath of sclerenchyma cells. Vascular tissue of grasses is enclosed by a layer of parenchyma called "bundle sheath cells," which, in $C_4$ plants, contain many large chloroplasts (Figure 2.7b).

## REPRODUCTIVE STRUCTURES

Flowers are organs unique to angiosperms. They are incredibly diverse in morphology and range from very showy

and conspicuous to very bland and almost imperceptible. They may occur singly or be arranged in different types of inflorescences (multiple flowers on a common axis). If a plant has male flowers (staminate) and female flowers (pistillate) on one plant, we call the plant "monoecious." If male flowers exist on one plant, and female flowers on another, we call the plant "dioecious" (two houses). Meiosis and alternation of generations occur in the ovule (female) and the anther (male). The male and female gametophytes are greatly reduced, consisting only of a few cells.

For the sake of simplicity, we will only consider perfect flowers (all flower parts, including both male and female structures, are present). The four basic units in a flower are the sepals, petals, stamens, and pistils (Figure 2.8a–c). The floral parts are arranged as whorls on the receptacle (Figure 2.8b). Working from the outside, inward at the base of the receptacle, the sepals, usually green, leaf-like structures, are the first whorl encountered, and these are collectively termed the "calyx." The next layer is composed of the petals, which can be green but are generally white or colored. The petals are usually larger than the sepals and are collectively termed the "corolla." The petals and sepals, when taken together, are also called the "perianth." In some cases, when the sepals and the petals are similar or almost indistinguishable, they are called "tepals" (Figure 2.8c). The stamens

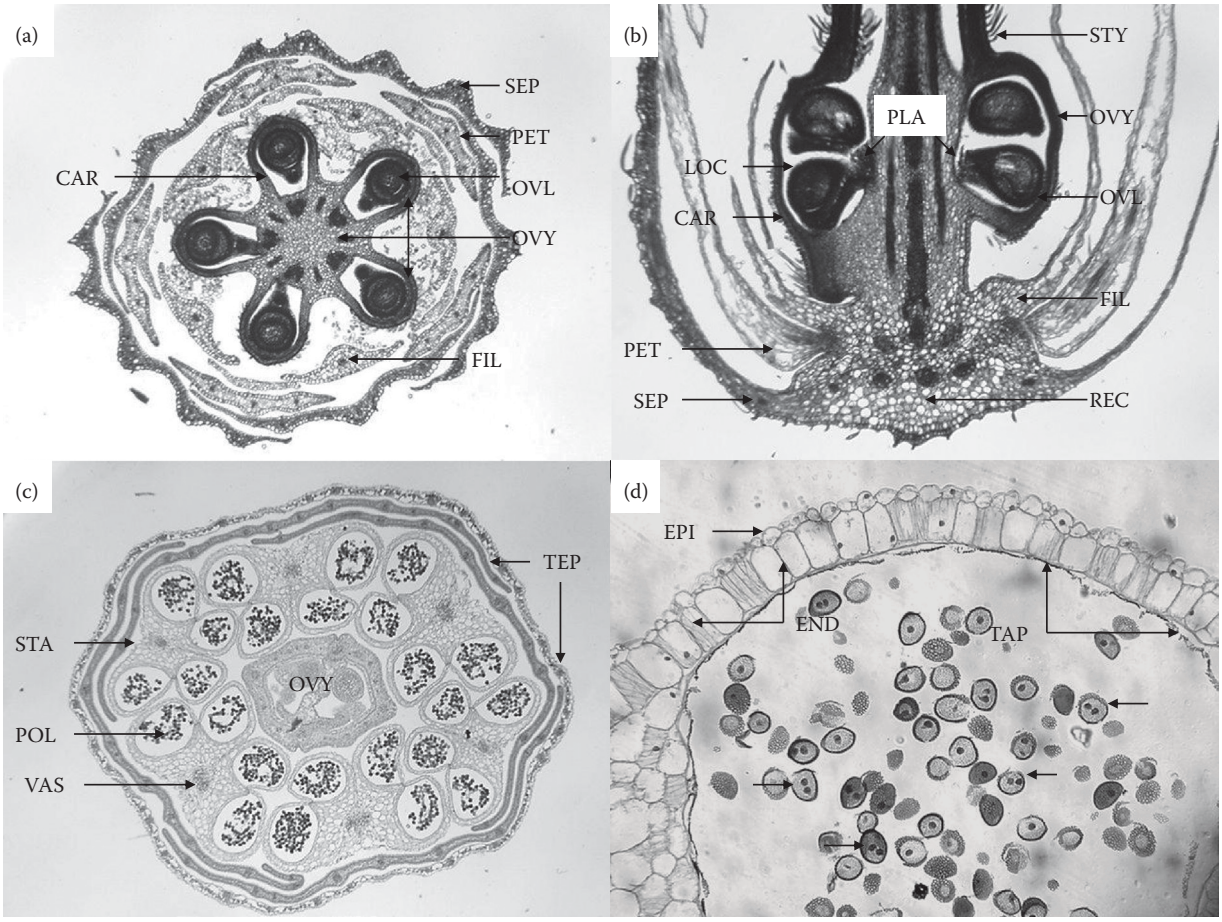


**FIGURE 2.8** Flower anatomy. (a) Cross section through a *Geranium* species (Stork's bill) flower. Progressing from the outside inward, sepals (SEP), petals (PET), and filaments (FIL: the stalks of the anther) are borne. Occupying the center of the flower is the ovary (OVY), which is composed of five carpels (CAR) in which two ovules (OVL) are borne. Note that only a single ovule can be seen in each locule (space) with this section. (b) Longitudinal section through the Stork's bill flower seen in (a). In this view, two carpels (CAR) and locules (LOC) of the ovary (OVY) are visible, but each contains only two ovules (OVL) of which only one will mature placenta. Note the trichomes (hairs) on the style (STY) portion of the ovary. The sepals (SEP), petals (PET), and filaments (FIL) are inserted below the ovary on the receptacle (REC). (c) Cross section of a lily (*Lilium* species) flower. In this view, six stamens (STA) containing pollen (POL) are shown. The vascular tissue (VAS) in the filament and four lobes of each anther are evident. Sepals and petals are collectively termed "tepals" (TEP) for lily flowers. OVY = ovary. (d) Higher magnification of an anther of lily. The anther wall is composed of the epidermis (EPI), the endothecium (END), and the tapetum (TAP), which, in this case, has degenerated and is only represented by the remnants of the cells. Many pollen grains are present—some in the binucleate stage (arrows). (c and d courtesy of Carolina Biological Supply, Burlington, North Carolina.)

or the male portion of the flower are the next set of structures found in the flower. A stamen consists of the filament or stalk (Figure 2.8a, b) that terminates in the anther, which contains pollen (Figure 2.8c, d). The pistil or female structure, which is more or less flask-shaped, occupies the center of the flower and consists of a swollen base, the ovary, a smaller-diameter stalk, the style, which ends in a somewhat swollen tissue, the stigma (Figure 2.8b; stigma not shown). Dicots typically have sepals, petals, and stamens in whorls of four or five, or multiples of four or five (Figure 2.8a), whereas monocots generally have these structures in groups of three or multiples of three (Figure 2.8c). Pistils may be composed of a single carpel (a structure analogous to a leaf rolled along the long axis, bearing ovules on the inner surface) or of many carpels (Figure 2.8a). One or more ovules may be attached to a common surface or placenta in each carpel (Figure 2.8b). Microspore mother cells (2n) in the anther and one megaspore mother cell (2n) in each ovule undergo meiosis (a reduction division), followed by mitosis, to form the male and the female gametophytes, respectively. When mature, the male gametophyte consists of three cells or nuclei, whereas the female gametophyte typically has eight cells or nuclei. The gametophytes are often placed in tissue culture in hopes of obtaining haploid plants. One must be cautious since many diploid plants may arise from the tapetum, the inner layer of cells lining the anther (Figure 2.8d) or the nucellus, which is a maternal cell layer delimiting the female gametophyte.

Pollination occurs when pollen (containing the male gametophyte) is transported via wind, insect, or other vector to the stigma. If the pollen is compatible with the female tissue and the stigma is receptive, the pollen grain will germinate and the germ tube will grow downward through the style toward the female gametophyte in the ovule. The pollen tube contains two sperm nuclei (each n) of which one fuses with the polar nuclei to form the primary endosperm nucleus (3n or other polyploid number), while the other unites with the egg (n) to form the zygote (2n). Thus, the sporophytic phase (2n) is restored. The primary endosperm nucleus divides and produces endosperm, and the zygote divides, producing the suspensor and the embryo. The embryo develops into a bipolar structure, possessing both shoot and root meristems and exhibiting bilateral symmetry. The dicot zygotic embryo has two cotyledons (Figure 2.9a), and the monocot embryo has one cotyledon (scutellum; Figure 2.9b). The integuments (seed coat) covering the ovule harden, and the ovule is now a seed (Figure 2.9a). The endosperm may be completely absorbed by the embryo or may remain outside the embryo.

Gymnosperms produce seeds, but do not flower, and with the exception of several non-coniferous gymnosperms, the cone is the primary reproductive structure.

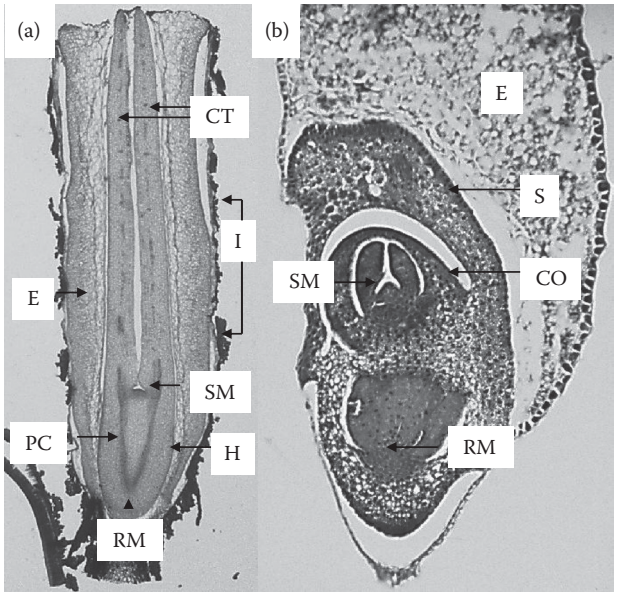


**FIGURE 2.9** Zygotic embryos. (a) Near-median longitudinal section through an immature seed of the dicot *Cercis canadensis* (redbud). Note the well-developed pair of cotyledons (CT), shoot meristem (SM), and hypocotyl (H); the root meristem (RM) is inconspicuous. E = endosperm; I = integument; PC = provascular tissue. (b) Near-median longitudinal section through a seed of the monocot *Dactylis glomerata* (orchardgrass). The shoot meristem (SM) and root meristem (RM), the single cotyledon (S = scutellum), and the first leaf (CO = coleoptile) are well developed. E = endosperm. (With kind permission from Springer Science+Business Media: *Protoplasma*.)

Microsporangiate cones (male) and megasporangiate (female) cones usually occur on the same plant. Each cone consists of an axis supporting a spiral of overlapping scales. On the female cone, two ovules develop on each scale within which the megaspore mother cell undergoes meiosis to produce four megaspores (n). Only one of these develops and, over the course of a year, grows to thousands of cells in size before producing two archegonia at one end, each containing an egg cell. From the male cone, pollen production and gametophyte development occur in a manner similar to angiosperms. However, once the pollen tube germinates, one of the sperm fuses with the egg to produce the zygote (2n), and there is no endosperm produced. Here, the seed coat and the thin layer of tissue to the inside of it originate from the female parent (2n), but the tissue that surrounds the zygote is megagametophyte (n). The embryo develops as in angiosperms, but with three to six cotyledons.

In ferns, no seeds are produced, and the alternation of generations is more apparent, with the gametophyte being a free-living haploid (n) stage known as a "prothallus." The prothallus is generally 2 to 20 mm in diameter and composed entirely of parenchyma cells. These are variously shaped, but are often flat and heart-shaped,

with thin rhizoids to serve the function of the roots. They may contain chlorophyll and grow on the soil surface, or be colorless and dependent on mycorrhizal associations. The prothallus produces cup-shaped archegonia (female), inside which a single egg is produced, and antheridia (male) that produce numerous motile sperm. A single prothallus may produce both structures, or they may occur on separate individuals. Water is required for the sperm to reach the egg and for fertilization to occur. The resulting zygote grows into the familiar sporophyte generation (2n). When mature, clusters of sporangia called "sori" are produced on specialized fronds, or in rows or along the margins of regular fronds. Within the sporangia, the spore mother cell undergoes two meiotic divisions to create a tetrad of spores (n) that are shed and germinate to grow into the prothallus. Numerous variations on this cycle exist.

## CONCLUSIONS

We have presented a very brief account of the anatomy of angiosperms, gymnosperms, and ferns, and we hope that we have whetted your appetite to further your study of this area of plant science. The study of plant anatomy allows us to understand more fully the process of plant development from the union of the egg and the sperm to the formation of the zygotic embryo to the development of the various tissues in the embryo and the mature plant—fascinating processes. The study of anatomy also allows us to somewhat understand the origin and the development of tissues from various propagules, including rooted cuttings and grafting.

If there is a laboratory associated with your course, we suggest that the following microscope slides (one supplier is Carolina Biological Supply, Burlington, North Carolina) may be useful for students to study:

- Dicot & Monocot Roots c.s. (30-1898)
- Typical Monocot & Dicot Stems c.s. (30-2642)
- Privet Leaf (Typical Dicot) *Ligustrum* c.s. (30-3838)
- Corn Leaf (Typical Monocot) *Zea* c.s. (30-4054)
- Typical Monocot & Dicot Flower Buds c.s. (30-4258)
- Shepherd's Purse Mature Embyo *Capsella* l.s. (30-4888)

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# 3 Plant Physiology Concepts Important for Propagation Success

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**Concept Box 3.1**

- An understanding of both the anatomy and the physiology of plants is critical for successfully propagating plants whether by sexual or asexual propagation techniques.
- Vegetative (asexual) propagation and sexual propagation differ in their ability to give rise to true-to-type progenies.
- Conceptually, each plant cell is endowed with the genetic information to potentially divide, grow, and differentiate into specialized cells and tissues, leading to a fully functional organism. This concept is referred to as "totipotency."
- Meristems are stem cells of plants; they proliferate, regulate, and direct plant growth.
- Plant propagation principles are well established, but their application is species and often individual genotype and growth stage dependent.
- Rooting of cuttings is one of the most common forms of plant propagation and success can be influenced even before the cutting is taken as a result of how the stock plant is grown and treated.
- Natural plant hormones interact and shape growth, differentiation, dedifferentiation, and many physiological events during their vegetative and sexual growth cycles. Propagators can manipulate these and even apply synthetic plant growth regulators to induce the responses that they desire.
- Some plant species are recalcitrant to propagation, and numerous combinations of genotype, media, hormones, and environment are used by plant propagators to overcome recalcitrance.
- Juvenile plants respond more readily to cutting propagation than do mature plants.
- Propagators use various techniques to "rejuvenate" stock plants and increase the likelihood of rooting cuttings. These include severe pruning, serial propagation onto juvenile seedlings, and the use of micro-propagated plants.
- Techniques such as blanching, etiolation, banding, and stool bed layering all work via the reduction of light exposure to enhance rooting.

Plant physiology is the study of how plants function, and an understanding of the basic plant physiological principles helps explain the success or the lack of success of the various techniques and practices used in plant propagation. A multitude of genetic or physiological factors influence plant response, whether the propagation occurs in the field, greenhouse, or in the laboratory. Metabolic and physiological processes in the plant are profoundly influenced by the environment of the plant, including light (intensity; day length; and quality, including light sources and wavelength differences), temperature, water relations, photoperiod, and season. A plant propagator must make decisions about the techniques to be employed, and these will be influenced by the type of plant that is being propagated (annual, biennial, or perennial) and whether a clone is desired. A clone is an offspring that is genetically identical to the parent and produced with mitotic divisions of cells and not meiosis (Chapter 5). The use of

cloning goes back to primitive agriculture, where pieces of woody stems were inserted into the ground to root, thus producing new plants. Clonal propagation maintains the genetic integrity of propagules through generations; it is also utilized to bypass or reduce the juvenile period and the characteristics of the growth forms associated with it, such as thorniness, juvenile leaf morphology, and the vegetative or nonflowering state. Much more can be learned about juvenility in Chapter 6. Clones of selected seed-propagated plants allow the widespread exploitation of unique traits or characters. Through the process of budding or grafting, the traits of a single desirable tree or even a branch (with a sport or desirable mutation) can be vegetatively perpetuated. A wonderful example of this is the 'Delicious' apple, which originated as a chance seedling in Iowa and is now commonly budded or grafted onto rootstocks to retain its unique features. Foresters also use cloning to perpetuate valuable traits in trees and describe the original seedling as an "ortet" and use the term "ramet" to designate its vegetative offspring or clone.

The environment of a plant interacts with the genotype to shape the characteristics of the plant, referred to as its "phenotype." For example, clones grown in two parts of the same field, although genetically identical, may show differences in size mainly due to microclimatic or soil properties and reflect gross or subtle differences in water and mineral nutrient availability. Such differences are largely environmentally induced. Just as both genetics and environment interact to shape plants, successful propagation is also affected by both genetics and environment. Furthermore, not only intrinsic and environmental conditions may have a profound influence on the propagule after it is separated from the parent plant, but also conditions that exist(ed) for the donor or parent plant that is used as a source of the propagule or explant.

The influence of individual aspects of the environment can differ dramatically depending on how they interact and affect the processes within the plant. For example, light plays a myriad of roles with respect to propagation and for plant growth. Light drives photosynthesis, which provides energy and carbohydrates to permit the growth and regeneration of roots on cuttings. Reduction of light has a profound effect on the way plants grow, and in the extreme, the absence of light can result in the etiolation of the plant. Etiolated plants, with their characteristic lack of chlorophyll and elongated stems, have a greater tendency to form roots. Light, particularly red and far-red light, regulates the light-sensitive pigment, phytochrome, which, in some plants, determines whether or not seeds will germinate (Chapter 36) and plants will flower. Other photoreceptors are sensitive to blue light and are involved in phototropism. The length of light relative to the dark period or photoperiod is important in signaling plants what part of the year or season it is and when the time is right for budbreak, flowering, senescence, and dormancy. Some species are able to form roots on cuttings in only a very narrow seasonal window during the year.

The following sections give a brief summary of some basic metabolic processes and their regulation within the plant, and examples of how those processes affect propagation success.

## BASIC PLANT PROCESSES THAT IMPACT PROPAGATION SUCCESS

### Light and Carbohydrate Metabolism (Photosynthesis and Respiration)

Photosynthesis is the "light-harvesting process" in plants. In photosynthesis, carbon dioxide molecules are taken in through the stomata and reduced by the water molecules that are split using the energy of light impinging on the chlorophyll complex located in specialized cell organelles called "chloroplasts." This photosynthetic apparatus of most higher plants reduces $CO_2$ by using the electrons from the splitting of the water molecule and converts them into molecules of the simple sugar, glucose, while releasing oxygen to our environment (Figure 3.1). The glucose molecules act as stored energy to enable growth and differentiation. Longer-term storage of the carbon fixed during photosynthesis occurs when the monosaccharide glucose is converted to starch, a polysaccharide that consists of long chains or polymers of glucose, or it may be utilized in the synthesis of the polymer cellulose, in forming cell wall lignin, or in a plethora of compounds utilized in cellular functions. Photosynthesis and respiration are basically reciprocal processes in a plant. In respiration, the plant uses the glucose and oxygen to release stored energy, water, and carbon dioxide. Although photosynthesis requires light and takes place in the chloroplasts, respiration can occur in the light or the dark, and occurs in the cytoplasm and the mitochondria of cells irrespective of their location. Photorespiration is also unique and especially deleterious to some angiosperms. Having adequate carbohydrate reserves in the form of

Photosynthesis

$$6\,CO_2 + 6\,H_2O + \text{energy} \xrightarrow{\text{(from light)}} C_6H_{12}O_6 + 6\,O_2$$

Aerobic respiration

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + \text{energy}$$

**FIGURE 3.1** Equations for photosynthesis depicting the fixation of carbon dioxide, with addition of water and light to form glucose and release oxygen. Aerobic respiration is the reciprocal of that for photosynthesis.

sugars and starches is important not only for the cutting that needs these resources to generate new roots, but also for the seeds that must have sufficient stored reserves to allow the germination and establishment of the seedling until it becomes photosynthetically competent.

Temperature can affect both photosynthesis and respiration. Photosynthesis typically increases with an increase in temperature up to an optimum (usually within the range of 25–30°C) and then declines more rapidly after that. Respiration typically doubles for every 10° increment over the range of 5 to 35°C (Talling, 1961) until the temperature exceeds the point where plants respire more than what the photosynthesis can compensate or under even higher temperature conditions where cellular enzymes begin to denature. Exact temperature optima can vary with the native environment of the species, the stage of the life cycle, and even the part of the plant. Temperature may have an effect on propagation success, for example, if stock plants experience high nighttime temperatures, the increased respiration can reduce carbohydrates stores, and this will have a negative impact on the rooting ability of the cuttings taken from the stock plants. In another case, it is customary to provide bottom heat when rooting evergreen cuttings in late fall or hardwood cuttings over the winter. The bottom heat provides moderate temperatures for the root zone, promoting callusing and adventitious root formation. The air temperature around the exposed portion of the cutting remains cool and keeps top growth from occurring too soon for the newly formed root system to support it.

## Light and Photoperiod Effects on Flowering

Light exerts effects on flowering and seed production, important to the plant breeder and the plant propagator. This effect is termed "photoperiodism." The name is somewhat of a misnomer because it is not so much the period of light that is important, but the length of the uninterrupted dark period. Plants that are responsive to day length are called either "long-day plants" or "short-day plants." Short-day plants, like chrysanthemums and poinsettias, flower only if the uninterrupted night period is longer than a certain critical length (Figure 3.2). Under long-day conditions, long-day plants, like clover, flower if the night period is shorter than a critical period. Plants responsive to day length are further divided into those that are obligate or facultative. Obligate short-day (like African marigold) or long-day (like dill or flax) plants must have the critical night period satisfied before they will flower, whereas facultative short-day (like dahlia) or long-day (like lily) plants will flower eventually once they reach a certain size, but the flowering response is hastened or enhanced by having the critical day length conditions satisfied.

Growers can create the right conditions for flowering for long-day plants even during naturally short-day conditions by extending the day length at the end of the day, a technique known as "day extension," or by providing a 4-h exposure to light in the middle of the dark period, known as "night interruption." Short-day conditions are typically created by pulling black cloth over

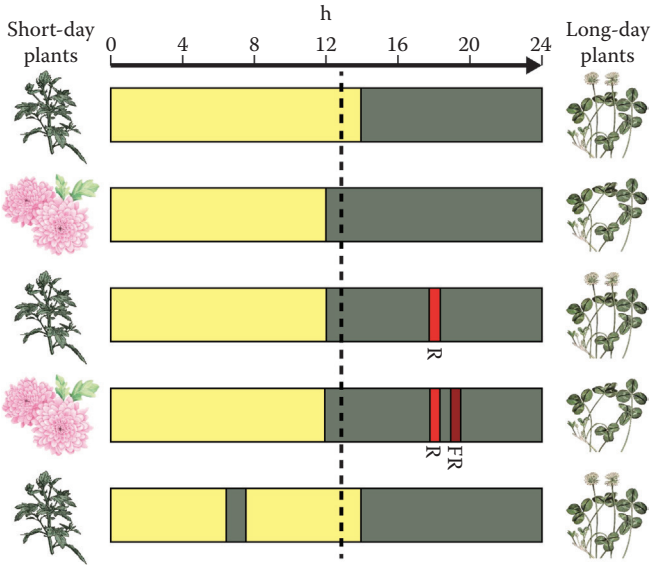


**FIGURE 3.2** In the top bar, long-day (short-night) conditions promote flowering for the long-day clover and keep the chrysanthemum vegetative. In the next bar, chrysanthemum flowers are under short-day (long-night) conditions, and the clover remains vegetative. A night break causes short-day conditions to exert the same effect as long days by converting a long night into two short nights. Far-red light (FR) exposure after red light (R) exposure reverses the outcome due to its effect on phytochrome (see Figure 3.3). A dark break provided in the middle of the light period does not affect flowering, indicating that it is the length of uninterrupted night that is critical to flowering responses.

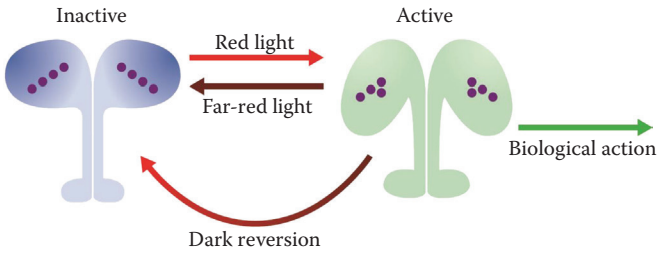


**FIGURE 3.3** Phytochrome exists in two forms. The inactive Pr form converts to the active Pfr form upon exposure to red light. This form is the one that triggers biological responses that are sensitive to photoperiod. The active form, Pfr, converts back to the inactive form with exposure to far-red light or slowly through dark reversion. (The shape of the phytochrome molecule was adapted from Smith, H., *Nature* 400:710–713, 1999.)

the plants to create a longer night inductive of flowering. Providing a dark break during the long light period has no impact on flowering since it is the length of the uninterrupted dark period that regulates the flowering response (Figure 3.2).

Photoperiodism is regulated by the light-sensitive pigment "phytochrome" that exists in two forms: an inactive form Pr and an active form Pfr (Figure 3.3). The red form, Pr, is maximally sensitive to 660-nm wavelength light, and the far-red form, Pfr, is maximally sensitive to 730-nm light. With exposure to red light (as you would have in bright daylight), the Pr form is converted to Pfr, which is the form that triggers light-sensitive responses in the plant. Exposure to far-red light (as you would have predominate in shade or in the evening) causes the Pfr form to revert back to the Pr inactive form. In the dark, there is a slow reversion back to the inactive form. Phytochrome has been implicated in the following functional categories of genes either as an agent of induction or repression: transcription, metabolism, photosynthesis/chloroplast, signaling, transport, growth/development, hormones, and stress/defense (Tepperman *et al*., 2006).

## Exclusion or Reduction of Light to Promote Rooting

Propagators have used various techniques to reduce light to precondition stock plants for better rooting responses. Various types of layering (Chapter 24), including air layering, simple layering, mound layering, and stool bed layering, all involve reduction or exclusion of light to some degree to enhance adventitious rooting. Technically, the term "etiolation" means growing plants without light, but to a nurseryman, etiolation means covering stock plants so that the new shoots emerge and grow under very heavy shade or in the darkness (Maynard and Bassuk, 1988). For some species, as little as a 50% reduction of light is effective to promote adventitious rooting as in the case of three Dahlia cultivars, increasing rooting from 7% to 75% (Biran and Halevy, 1973). Reducing the irradiance of stock plants has promoted rooting in such diverse plant species such as pea (Bertram and Veierskov, 1989), juniper (Lin and Molnar, 1980), aspen and willow (Eliasson and Brunes, 1980), and apple (Christensen *et al*., 1980).

When the shade or light-impervious cover is removed, the base of etiolated shoots is often covered with an opaque band such as an ordinary black, insulating tape or even Velcro bands (Figure 3.4). It is important that the tissues that are banded be as undifferentiated or close to the tip as possible (Gardener, 1937). Applying a wide band is more effective than using a narrow band. With *Carpinus betulus* "Fastigiata," rooting of 87% was obtained with a 7.5-cm band and much less with bands that were thinner (63% with a 5-cm band and 53% with

**FIGURE 3.4** Etiolated shoots of cut back oak hybrids before being treated with growth regulator to promote roots. Note the characteristic lack of chlorophyll; elongated shoots; small leaves; and soft, succulent stem tissue. (Courtesy of Nina Bassuk.)