

生物实验室系列

# Arabidopsis a Laboratory Manual

## 拟南芥实验手册

(英文影印版)

Detlef Weigel and Jane Glazebrook



**Chemical Industry Press**



化学工业出版社  
现代生物技术与医药科技出版中心

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# ARABIDOPSIS

*A Laboratory Manual*

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**Arabidopsis: A Laboratory Manual**

拟南芥实验手册

Detlef Weigel, Jane Glazebrook

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## 出版者的话

21 世纪是生命科学的世纪，这已成为人们的共识。

生命科学随着人类对自身和自然的认识、探索而萌芽，随着人类生产和科学实践的进步而发展。现代生命科学包括生物学、医学、农学等传统学科领域，以及生物学、生物技术与环境科学乃至社会科学等其他学科相互渗透、交叉而产生的新型学科体系。20 世纪后叶现代生物科学尤其是分子生物学取得了一系列突破性成就，使得生命科学在自然科学体系中的位置发生了革命性的变化，成为 21 世纪的带头学科。人们对生命科学也寄予了无限的期望，希望能够解决人类社会所面临的人口膨胀、资源匮乏、疾病危害、环境污染和生态破坏等一系列重大问题。

回顾生命科学的发展历程，实验技术一直起着非常重要的促进作用。如 17 世纪 Leeuwenhoek 等人发明并应用显微镜技术，直接催生了“细胞学说”的建立和发展；1973 年 Cohn 和 Boyer 完成了 DNA 体外重组实验，标志着基因工程的肇始；1988 年 Kary Mullis 发明的 PCR 技术甚至使生命科学产生了飞跃性的发展。可以说，生命科学无时无刻离不开实验，实验是开启神奇的生命王国大门的钥匙。没有实验技术的不断进步，也就没有生命科学今天的巨大发展；同时，生命科学的发展又对实验技术提出了更高的要求，进一步刺激了后者的不断进步。生命科学正是在“实验催生和验证着基础理论，理论指导和发展的实验技术”的不断循环中从必然王国走向自由王国。

工欲善其事，必先利其器。为了有助于生命科学工作者更多地了解相关实验技术和仪器设备，更好地设计实验方案，更有效地开展实验过程，更合理地处理实验结果，化工出版社组织出版了“生物实验室系列图书”。系列图书在整体规划的基础上，本着“经典、前沿、实用，理论与技术并重”的原则组织编写，分批出版。

在题材上，系列图书涵盖综合实验技术和单项实验技术两个方面。其中综合实验技术既有以实验目的为题，如“蛋白质化学分析技术”，内容纵向覆盖多项实验技术；也有以某一生命学科领域的综合实验技术为题，如“发酵工程实验技术”、“生物化学实验技术”等。

而单项实验技术则以深入介绍某一专项技术及其应用为主，在阐述其基本原理的基础上，横向介绍该项技术在多个领域的应用，如“双向电泳技术”、“流式细胞术”等。

**在内容上，系列图书主要有以下两个显著特点。**一是强调先进性——除了系统介绍常用和经典实验技术以外，特别突出了当前该领域实验手段的新理论、新技术、新发展，为国内专业人员起到借鉴和引导作用。二是强调可操作性——对于每一项实验技术，系统介绍其原理方法、设备仪器和实验过程，让读者明了实验的目的、方案设计以及具体步骤和结果处理，以期起到实验指南的作用。

**本系列图书坚持质量为先，开拓国内和国际两个出版资源。**一方面，约请国内相关领域兼具理论造诣和丰富实验室工作经验的专家学者编著；另一方面，时刻关注国际生命科学前沿领域和先进技术的进展，及时引进（翻译或影印）国外知名出版社的权威力作。

“生物实验室系列图书”的读者对象设定为国内从事生命科学及生物技术和相关领域（如医学、药学、农学）的专业研究人员，企业或公司的生产、研发、管理技术人员，以及高校相关专业的教师、研究生等。

我们殷切希望“生物实验室系列图书”的出版能够服务于我国生命科学的发展需要，同时热忱欢迎从事和关心生命科学的广大科技人员不仅对已出版图书提供宝贵意见和建议，也能对系列图书的后续题目设计贡献良策或推荐作者，以便我们能够集思广益，将这一系列图书沿着可持续发展的方向不断丰富品种，推陈出新。

**谨向所有关心和热爱生命科学，为生命科学的发展孜孜以求的科学工作者致以崇高的敬意！**

**祝愿我国的科技事业如生命之树根深叶茂，欣欣向荣！**

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现代生物技术与医药科技出版中心

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# Preface

*Arabidopsis thaliana* has emerged as the model organism of choice for a wide range of basic and applied research into plant biology. This is largely due to the strength of *Arabidopsis* for genetic approaches, and, with the completion of its genome sequence, for genomic approaches as well. Our intention was to provide a simple guide to the application of *Arabidopsis* genetics to studies of plant biology. We hope that this volume will be a useful laboratory manual for those new to *Arabidopsis* genetics, as well as a reference for more established investigators when they require a commonly used protocol with which they are not familiar.

We have organized the manual in the order that a typical project proceeds. The early chapters cover growing *Arabidopsis*, identification of mutants, and basic genetic analyses. Later chapters cover phenotypic analyses, isolation of genes identified by mutations, and methods for studying gene expression. Appendix 1 provides Internet resources for the *Arabidopsis* community. We have concentrated on methods that are widely used in the community, in an attempt to provide a useful, yet affordable reference. We have not provided protocols for a large number of basic molecular biology methods that are not specific to *Arabidopsis* research. Readers who are not familiar with such methods should consult one of the many excellent molecular biology manuals.

The idea of writing this manual grew out of our experiences teaching the *Arabidopsis* summer course at Cold Spring Harbor Laboratory. We thought that the manual for that course would be useful for the wider community, but should be expanded to cover a broader range of methods. Daphne Preuss was instrumental in the early stages of plan-

ning and compiling the manual, and she contributed one of the chapters as well as several protocols. Other protocols were provided by us, members of our laboratories, or colleagues. We are very grateful to all contributors, without whom this manual could not have been produced. They are credited at the beginning of each protocol. Many of these protocols have been widely used for a long time, and we certainly do not pretend to have invented them, we merely wrote them down.

We are indebted to the excellent staff of Cold Spring Harbor Press. Mark Curtis drew the figures, Siân Curtis did a terrific job as developmental editor, making our text suitable for publication, and production editor Dorothy Brown made it into a book. Finally, without the persistence, patience, and organization of Kaaren Janssen and Mary Cozza, this project would never have been completed.

We hope that investigators of *Arabidopsis* will find this manual useful, and we welcome any feedback on the improvement of the protocols or any mistakes in them.

Detlef Weigel  
Jane Glazebrook

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# Contents

Preface, xi

## CHAPTER 1

### How to Grow *Arabidopsis*, 1

Morphology and Anatomy	1
Seed Collection and Storage	7
Cultivation of <i>Arabidopsis</i>	7
General Considerations	7
Growth Environment	8
Cultivation of Plants in Soil	10
Use of Solid Media	12
Other Growth Conditions	13
Pest Management	14
Supplier's Web Sites	17
References	18

## CHAPTER 2

### Obtaining Mutants, 19

Obtaining Previously Identified Mutants from the Stock Centers	19
Forward Genetics: Finding Mutations That Cause Particular Phenotypes	20
EMS Mutagenesis of Seed	24
T-DNA Mutagenesis	26
Special T-DNA Vectors	26
Reverse Genetics: Finding Mutations in Particular Genes	30
Screening DNA Pools for T-DNA Insertions	31
Considerations for Phenotypic Characterization	34
Beyond Mutants: Using Natural Variation to Identify Interesting Genes	35
References	37



## CHAPTER 3

## Genetic Analysis of Mutants, 41

Setting up Crosses	41
Choosing Parent Plants	41
Choosing Flowers	41
Segregation Analysis	43
Self-progeny	43
F <sub>1</sub> Progeny	44
F <sub>2</sub> Progeny	44
Backcrosses and Cosegregation	46
Complementation Testing	48
Strategies for Identifying Double Mutants	50
Finding Double Mutants in the F <sub>2</sub> Generation	50
Finding Double Mutants in the F <sub>3</sub> Generation	51
The Brute Force Approach	52
References	53

## CHAPTER 4

## How to Analyze a Mutant Phenotypically, 55

Growth Parameters	56
Quantitative Analysis of Root Growth	56
Hypocotyl Length	59
Flowering Time	60
Germination Rate	62
Fresh Weight Gain	62
Water Loss	62
Hormone Response	63
Gibberellin/Abscisic Acid/Paclobutrazol	63
Auxin	64
Ethylene	66
Brassinosteroids	69
Response to the Abiotic Environment	71
Root Elongation Under Salt/Hormone-induced Stress	71
Germination Rate	72
Electrolyte Leakage	73
Fresh Weight Gain	74
Water Loss	75
Others: Proline and Sugar Content	75
Heavy Metal Stress	75
Bacterial Pathogens	77
Preparation of Bacterial Cultures and Inoculation of Plants	77
Testing the Hypersensitive Response	78
Assessing Bacterial Growth	79
Oomycete Pathogens: <i>Peronospora parasitica</i>	81
Reviving Frozen Stocks and Inoculating Plants	81
Rating <i>Peronospora</i> Infections	83

Preparation of Frozen Tissue Sections	83
Maintaining Infections	84
Diaminobenzidine Stain for Hydrogen Peroxide	85
Trypan Blue Stain for Fungi, Oomycetes, and Dead Plant Cells	86
Histology	87
Preparation of Tissue Sections of Fixed Material	87
Specialized Staining Techniques	93
The PAS Reaction for Staining Cell Walls	93
Alcian Blue–PAS Reaction	95
Phloroglucinol Stain for Lignin	96
Vital Stain for Cytoplasm	97
Neutral Red Staining for Vacuoles	98
Whole-mount DAPI Staining and Measurement of DNA Content	98
Nuclear Staining for Confocal Microscopy	100
Cleared Tissue for Observation of Vascular Strands	104
Agarose Imprints of Surfaces	105
Scanning Electron Microscopy	106
Standard SEM Protocol	106
Dental Wax Impressions to be Viewed in SEM	109
SEM "Quick-and-Easy" Fixation	109
Imaging of Fresh <i>Arabidopsis</i> Tissues in the SEM	110
Transmission Electron Microscopy	112
Standard TEM Protocol	112
TEM Freeze Substitution	114
References	115

## CHAPTER 5

How to Transform <i>Arabidopsis</i> , 119	
Vectors and <i>Agrobacterium</i> Hosts	119
<i>Agrobacterium</i> strains	122
Transformation of <i>Agrobacterium</i>	122
Transformation of <i>Agrobacterium</i> Using Electroporation	123
Transformation of <i>Agrobacterium</i> Using the Freeze-Thaw Method	125
PCR Analysis of <i>Agrobacterium</i>	127
In Planta Transformation of <i>Arabidopsis</i>	128
Plant Growth	128
Floral Dip of <i>Arabidopsis</i>	129
Alternative Protocol 1: Vacuum Infiltration	130
Alternative Protocol 2: Spraying	131
Selecting Transformed Plants	131
Kanamycin Selection	131
Basta Selection on Soil	133
Root Transformation	134
References	140

## CHAPTER 6

How to Isolate a Gene Defined by a Mutation, 143	
Isolating a Gene Defined by an Insertion Mutation	143
TAIL-PCR	144
Isolating a Gene Known Only by the Phenotypes of Mutant	
Alleles: Positional Cloning	155
STEP 1: Determining the Approximate Map Position for <i>yfg</i>	155
STEP 2: Define the Map of <i>yfg</i> as Narrowly as Possible	161
STEP 3: Finding <i>YFG</i> within the Mapped Interval	162
Special Cases	162
Serious Errors	164
Purifying DNA from <i>Arabidopsis</i>	165
CTAB DNA Miniprep	165
Dellaporta DNA Miniprep	166
Quick DNA Prep for PCR	168
References	169

## CHAPTER 7

How to Study Gene Expression, 171	
RNA Expression	171
RNA Extraction for Northern Blots and RT-PCR	172
Semiquantitative Reverse Transcription followed by PCR	173
In Situ Hybridization to Tissue Sections	181
Radioactive In Situ Hybridization	182
Nonradioactive In Situ Hybridization	195
Whole-mount In Situ Hybridization	212
Protein Expression	215
Extraction of Total Protein for Western Blots	215
Organelle Preparations	216
FPLC Gel Filtration	226
Nondenaturing Gel Electrophoresis of Proteins	228
Protein Coimmunoprecipitation	233
In Situ Localization of Proteins	237
Reporter Genes	241
Whole-mount GUS Staining	243
Quantitative GUS Activity Assays	249
Subcellular Localization of GUS- and GFP-Tagged Proteins in	
Onion Epidermal Cells	252
Live-cell Imaging of GFP	262
Liquid LUC Activity Assays	267
LUC Imaging of Whole Plants	269
References	276

## CHAPTER 8

## How to Study Gene Function, 281

Reducing Gene Expression	282
Antisense RNA	282
Cosuppression/RNAi	284
Tissue- or Stage-specific Gene Knock-outs	286
Misexpression	287
Constitutive/Tissue-specific Promoter Fusions	287
Two-component Systems for Tissue-specific Misexpression	287
The <i>Alc</i> Gene Ethanol-inducible Switch system	291
Glucocorticoid Inducible Control of Gene Expression	293
Heat Shock Induction	295
Glucocorticoid Fusions for Transcription Factors	296
Transient Expression	300
Transient Expression in Protoplasts	300
Transgene Expression in Regenerated Roots	304
Other Gain-of-Function Strategies	308
Mosaic Analysis	309
References	313

## APPENDIX 1

Where to Find Information on <i>Arabidopsis</i>	317
---	-----

## APPENDIX 2

Critical $\chi^2$ Values	321
--------------------------	-----

## APPENDIX 3

Cautions	323
----------	-----

## APPENDIX 4

Suppliers	341
-----------	-----

## Index, 343

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# How to Grow *Arabidopsis*\*

The plant *Arabidopsis thaliana*, like most model organisms, is convenient and inexpensive to grow. It thrives in soil-filled pots and can be maintained in greenhouses, growth chambers, custom-made growth rooms, or even on window ledges and outdoors. The plants also grow readily in defined solid or liquid media, allowing rapid screening for seedling defects, recovery of transformants, and propagation of pathogen-free tissues. Parameters to consider for each of these growth environments are discussed in this chapter, and a brief introduction to the anatomy and morphology of *Arabidopsis* is given below.

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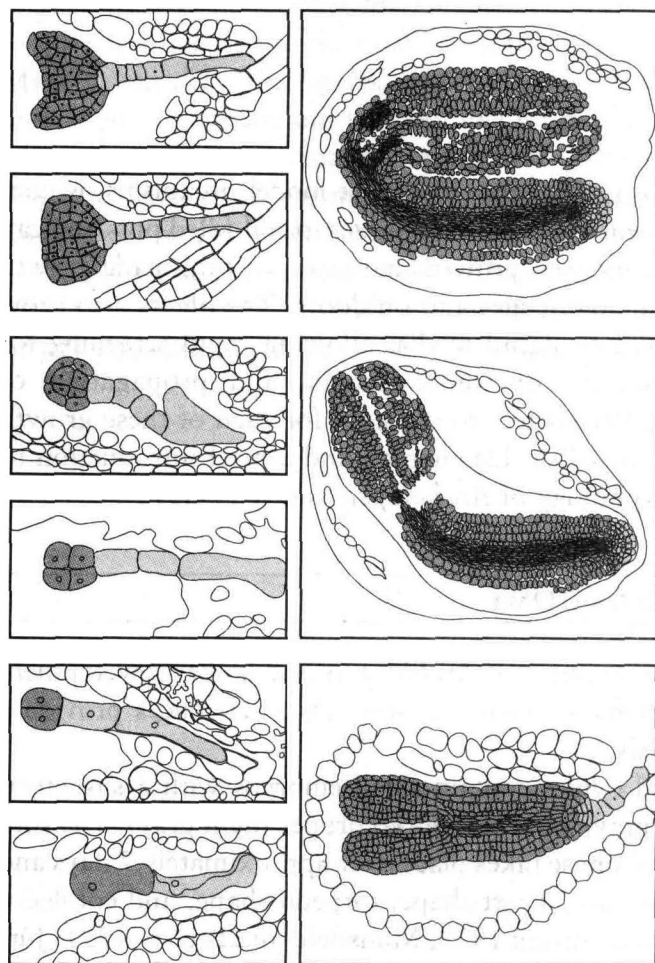
## MORPHOLOGY AND ANATOMY

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The major anatomical features of *Arabidopsis* and its key developmental landmarks are briefly summarized here; cited references provide a more thorough analysis.

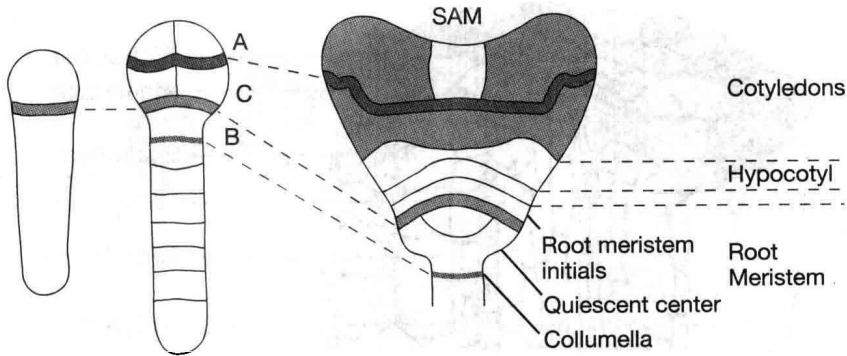
Recently fertilized ovules contain small embryos with a suspensor (see Figure 1.1, dark gray) and the embryo proper (light gray). The subsequent developmental phase takes place over approximately 7 days and is divided into the globular, heart-shape, torpedo-shape, and cotyledonous stages (Meinke and Sussex 1979; Mansfield and Briarty 1991; Jürgens and Mayer 1993). As the embryos mature, the seed pods (siliques) become yellow and then dry completely, finally shattering and releasing the seeds. The collection and storage of seeds are discussed later in this chapter.

\*Chapter contributed by Daphne Preuss (Howard Hughes Medical Institute, University of Chicago, Illinois).



**Figure 1.1.**

Embryogenesis of *Arabidopsis*. The zygote divides initially into an apical cell and a basal cell. (*Light gray*) The embryo proper, which is derived from the apical cell; (*dark gray*) the descendants of the basal cell, which give rise to the suspensor and the root meristem. (*Top row from left to right*) A two-celled embryo; the octant stage, with two tiers of embryonic cells; the dermatogen stage, during which the epidermal primordium (protoderm) is set aside; and the early heart stage, during which the cotyledon primordia emerge and the root meristem develops. (*Bottom row*) The late-heart stage, early and late torpedo stages, and the bent cotyledon stage. (Adapted, with permission, from C.-M. Liu, Plant Research International, Wageningen, The Netherlands and at <http://www.lsc.okstate.edu/chunming/plantembryo.html>)



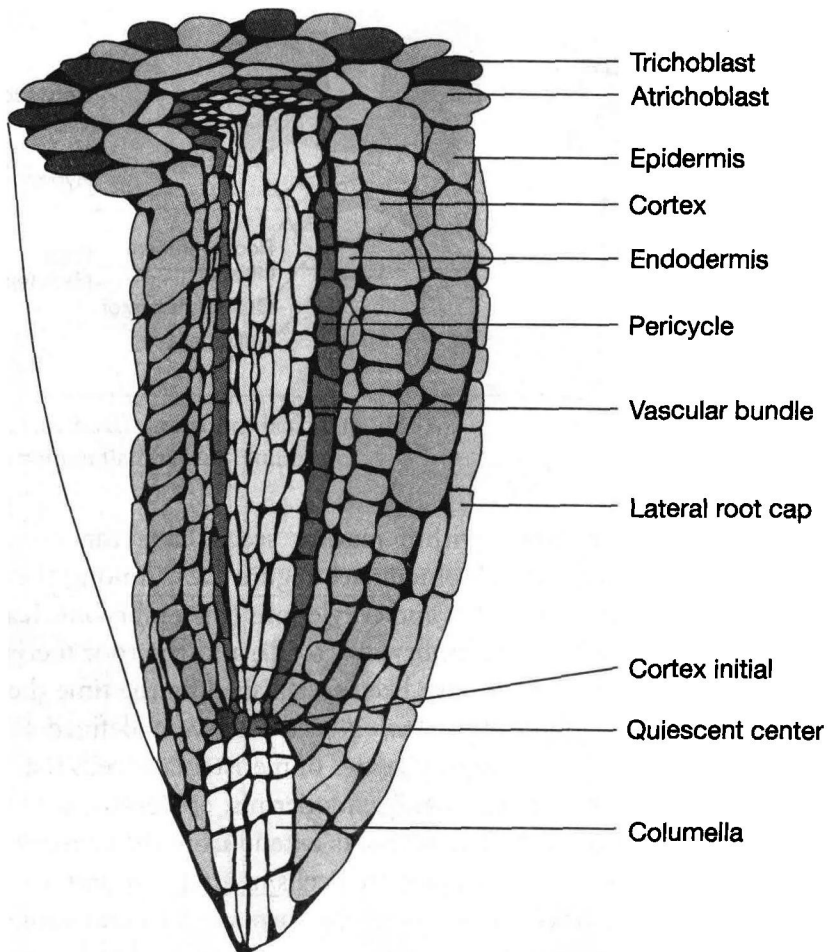
**Figure 1.2.**

Fate map of the early embryo. (SAM) Shoot apical meristem. (Redrawn, with permission, from Ben Scheres at <http://www.bio.uu.nl/~mcbroots/fatemap.htm>.)

The embryos contained within mature seeds have the necessary organs for early seedling development (see Figure 1.2). Among these are the shoot apical meristem (SAM) and cotyledons (the embryonic leaves), the hypocotyl, the radicle (the embryonic root), and the root meristem.

The key layers of the root have been established by the time the seed is mature. Root development in *Arabidopsis* follows a defined lineage (Dolan et al. 1993), with a quiescent center of meristematic cells that form the vascular bundle, pericycle, cortex, endodermis, epidermis, columella, and root cap (see Figure 1.3). Lateral roots extend from the primary root, and root hairs emerge from the epidermal cells in a regular pattern.

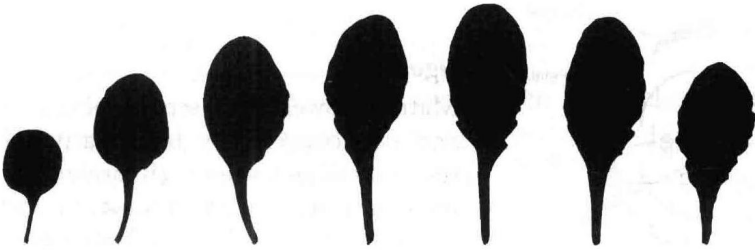
Shoot development continues with the formation of leaf primordia from the shoot apical meristem, which develop into rosette leaves. The basal part of the leaf, where it attaches to the stem, is called a petiole; the rest is made up of the leaf blade. The number of rosette leaves varies with growth conditions; short days favor rosette growth, whereas long days favor the initiation of reproductive development. The first two true leaves arise opposite each other and are more or less round. Subsequent leaves arise in a spiral and are more oval. Most commonly used ecotypes have leaves slightly serrated (Figure 1.4). On the leaves, hair-like projections (trichomes) serve as useful markers to distinguish juvenile and adult tissues (Kerstetter and Poethig 1998). The first few rosette leaves lack trichomes on their lower, or abaxial, surface. Subsequent leaves have trichomes on both surfaces. Leaves on the inflorescence shoot, also called cauline leaves or sometimes bracts, lack trichomes on their upper, or adaxial, surface.



**Figure 1.3.** \_\_\_\_\_  
Anatomy of root tip. See text for details.

In most laboratory strains such as Landsberg *erecta* (see Figure 1.5) or Columbia, floral meristems are initiated on the flanks of the primary shoot apical meristem about 2 weeks after germination, when plants are grown in long days. These meristems develop into floral buds, which become visible in the center of the rosette after approximately 1 week. Subsequently, the main shoot elongates and produces an inflorescence. The shoot elongation process is also referred to as bolting. Colloquially, the inflorescent shoot is often called a bolt, but this is not proper English (a bolt is part of a lock or a projectile or a measure of fabric, but never part of a plant!). Cauline leaves decorate the lower part of the



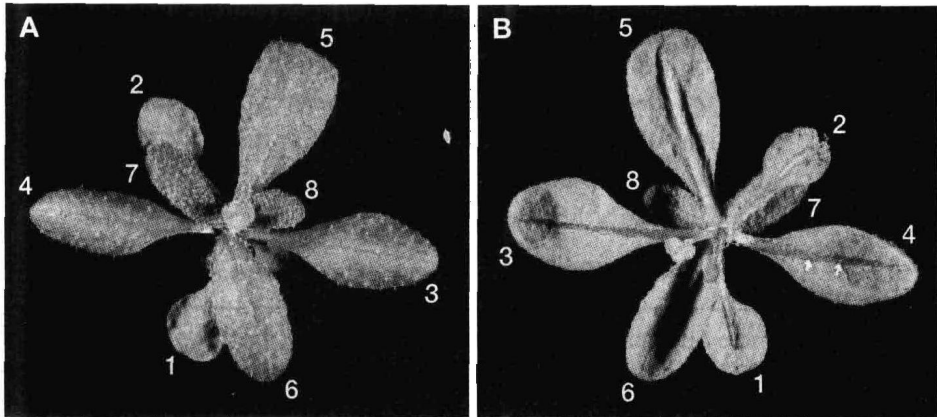


**Figure 1.4.**

Drawing of leaves two to eight (in order of appearance) in the Columbia ecotype. Note changing morphology of leaves, with overall shape changing from round to oval, and margins from entire to serrated. (Redrawn, with permission, from Kerstetter and Poethig 1998 © Annual Reviews.)

inflorescence shoot. As with the leaves, flowers form in a spiral pattern, with approximately two flowers opening each day. Flowers have four whorls of major organs, which are, from the outside to the inside, four sepals, four petals, six stamens, and two congenitally fused carpels that form the central gynoecium (see Figure 1.6) (Smyth et al. 1990).

Pollen development occurs within the anther locules; each *Arabidopsis* flower can produce up to 1000 pollen grains. A microspore mother cell undergoes meiosis and produces a tetrad of microspores; two subsequent mitotic divisions yield mature pollen grains containing a vegetative



**Figure 1.5.**

Rosette leaves in the Landsberg *erecta* ecotype. (Left) Upper leaf surfaces with trichomes on all leaves. Numbers indicate order of leaf appearance. (Right) Lower leaf surfaces on the same rosette, with trichomes gradually appearing, first on the midrib of leaf 3 and 4 (arrow), and only in large numbers from leaf 7 on. (Redrawn, with permission, from Telfer and Poethig 1998 © Company of Biologists Ltd.)