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Plant Developmental Biology

Methods and Protocols

Edited by

Lars Hennig

and

Claudia Köhler

Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

Humana Press

Editors
Lars Hennig
Department of Biology
Swiss Federal Institute of
Technology (ETH)
Universitätstrasse 2
CH-8092 Zurich
Switzerland
lars.hennig@ipw.biol.ethz.ch

Claudia Köhler
Department of Biology
Swiss Federal Institute of
Technology (ETH)
Universitätstrasse 2
CH-8092 Zurich
Switzerland
claudia.koehler@ipw.biol.ethz.ch

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Preface

Plants come in myriads of shapes and colors, and the beauty of plants has fascinated mankind for thousands of years. Long before Mendel discovered the laws of heritability and Darwin developed his theory on evolution, the affection for ornamental plants led people to select alleles that establish novel plant forms. Today, plant developmental biology tries to discover the mechanisms that control the establishment of specialized cell types, tissues, and organs from the fertilized egg during a plant's life. Although the underlying processes of cell proliferation and differentiation are similar in plants and animals, plants are different because their development is usually open, and its outcome is not the faithful repetition of a general plan but is strongly influenced by environmental conditions. In the last few decades, plant developmental biology has pinpointed a large number of developmental regulators and their interactions and the mechanisms that govern plant development start to emerge. In part, this progress was enabled by the advance of powerful molecular tools for a few model species, most importantly Arabidopsis.

This volume of the *Methods in Molecular Biology* series provides a collection of protocols for many of the common experimental approaches in plant developmental biology. All chapters are written in the same format as that used in the *Methods in Molecular Biology*™ series. Each chapter opens with a description of the basic theory behind the method being described. The Materials section lists all the chemicals, reagents, buffers, and other materials necessary for carrying out the protocol. Since the principal goal of the book is to provide experimentalists with a full account of the practical steps necessary for carrying out each protocol successfully, the Methods section contains detailed step-by-step descriptions of every protocol that should result in the successful completion of each method. The Notes section complements the Methods material by indicating how best to deal with any problem or difficulty that might arise when using a given technique. Reflecting the current balance in the field, the book is most detailed for Arabidopsis but includes also protocols for other model species such as rice, maize, or Medicago. The book is divided into six major parts: growth protocols, manipulation of gene activity, assaying developmental phenotypes, assaying gene activity, testing protein–protein interactions, and probing chromatin. Presented methods are diverse and range from grafting over bimolecular fluorescence complementation to chromatin immunoprecipitation. In the first place, the book addresses a target audience of plant developmental geneticists and biochemists. In addition, colleagues from other fields such as stress physiology or plant nutrition will find this book helpful. Developmental biology was usually not the prime interest of these colleagues, but when analyzing mutants, which are nowadays so easily available using reverse genetics, many researchers will suddenly be confronted with phenotypes of abnormal development. Together, we hope that this volume will be an essential part of many laboratory libraries. We would be pleased if the book will be found more often on the bench top than in the book shelf.

L. Hennig
C. Köhler

Contents

Contributors

ERNST AICHINGER • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*

JOSÉ AKER • *Laboratory of Biochemistry, Wageningen University, Wageningen, Netherlands*

CRISTINA ALEXANDRE • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*

GERRIT T.S. BEEMSTER • *Department Plant Systems Biology, Flanders Institute for Biotechnology & Department Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium; Department of Biology, University of Antwerp, Antwerp, Belgium*

PHILIP N. BENFEY • *Department of Biology and NIH Center for Systems Biology, Duke University, Durham, NC, USA*

STEVEN BERNACKI • *Department of Plant Biology, North Carolina State University, Raleigh, NC, USA*

LORENZO BORGHI • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*

JAN WILLEM BORST • *Laboratory of Biochemistry, Wageningen University, Wageningen, Netherlands*

CHRISTOPH BÜCHERL • *Laboratory of Biochemistry, Wageningen University, Wageningen, Netherlands*

CRISTEL C. CARLES • *Plant Gene Expression Center, USDA-ARS/UC Berkeley & Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA*

SARAH J. COOKSON • *Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux UMR759, INRA-SUPAGRO, Montpellier, France*

FREDERIK COPPENS • *Department Plant Systems Biology, Flanders Institute for Biotechnology & Department Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium*

TAMAS DALMAY • *School of Biological Sciences, University of East Anglia, Norwich, Norfolk, England*

ROBERT C. DAY • *Department of Biochemistry, University of Otago, Dunedin, Otago, New Zealand*

HANS J. DE JONG • *Laboratory of Genetics, Wageningen University, Wageningen, Netherlands*

SACCO DE VRIES • *Laboratory of Biochemistry, Wageningen University, Wageningen, Netherlands*

STIJN DHONDT • *Department Plant Systems Biology, Flanders Institute for Biotechnology & Department Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium*

VIVIEN EXNER • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*

FABIO FIORANI • *Department Plant Systems Biology, Flanders Institute for Biotechnology & Department Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium*

ELISA FIUME • *Plant Gene Expression Center, USDA-ARS/UC Berkeley & Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA*

JENNIFER C. FLETCHER • *Plant Gene Expression Center, USDA-ARS/UC Berkeley & Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA*

Paul Fransz • *Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands*
Jiří Friml • *VIB Department of Plant Systems Biology, University of Ghent, Ghent, Belgium*
Christine Granier • *Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux UMR759, INRA-SUPAGRO, Montpellier, France*
Rita Groß-Hardt • *Center for Plant Molecular Biology (ZMBP), University of Tübingen, Tübingen, Germany*
Chan Man Ha • *Plant Gene Expression Center, USDA-ARS/UC Berkeley & Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA*
Renze Heidstra • *Molecular Genetics Group, Department of Biology, Utrecht University, Utrecht, Netherlands*
Lars Hennig • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*
Pierre Hilson • *Flanders Interuniversity Institute for Biotechnology (VIB), Department of Plant Systems Biology, Ghent University, Ghent, Belgium*
Martin Hülskamp • *Botanical Institute III, University of Cologne, Cologne, Germany*
Erika Isono • *Department of Plant Systems Biology, Technical University Munich – Weihenstephan, Munich, Germany*
Anjali S. Iyer-Pascuzzi • *Department of Biology and NIH Center for Systems Biology, Duke University, Durham, NC, USA*
Mark A. Johnson • *Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI, USA*
Jason Jones • *Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA*
Ji Hyung Jun • *Plant Gene Expression Center, USDA-ARS/UC Berkeley & Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA*
Mansour Karimi • *Flanders Interuniversity Institute for Biotechnology (VIB), Department of Plant Systems Biology, Ghent University, Ghent, Belgium*
Naohiro Kato • *Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA*
Jin Sun Kim • *Plant Gene Expression Center, USDA-ARS/UC Berkeley & Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA*
Stefan Kircher • *Albert-Ludwigs-University of Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany*
Claudia Köhler • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*
Benedikt Kost • *Uppsala BioCenter, Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Ultuna, Uppsala, Sweden*
Thomas Kretsch • *Albert-Ludwigs-University of Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany*
Catherine Massonnet • *Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux UMR759, INRA-SUPAGRO, Montpellier, France*
Yvonne Möller-Steinbach • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*
Nir Ohad • *Department of Plant Sciences, Tel-Aviv University, Tel-Aviv, Israel*
Penka Pavlova • *Laboratory of Genetics, Wageningen University, Wageningen, Netherlands*

SERENA PERILLI • *Dipartimento di Genetica e Biologia Molecolare, Laboratory of Functional Genomics and Proteomics of Model Systems, Università La Sapienza, Rome, Italy*
HELENA R. PIRES • *Plant Gene Expression Center, USDA-ARS/UC Berkeley & Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA*
NIKI ROBERTSON • *Department of Plant Biology, North Carolina State University, Raleigh, NC, USA*
BART RYMEN • *Department Plant Systems Biology, Flanders Institute for Biotechnology & Department Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium*
SABRINA SABATINI • *Dipartimento di Genetica e Biologia Molecolare, Laboratory of Functional Genomics and Proteomics of Model Systems, Università La Sapienza, Rome, Italy*
MICHAEL SAUER • *Centro Nacional de Biotecnologia CSIC Madrid, Madrid, Spain*
CLAUS SCHWECHHEIMER • *Department of Plant Systems Biology, Technical University Munich – Weihenstephan, Weihenstephan, Germany*
RÜDIGER SIMON • *Institute of Genetics, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany*
YVONNE STAHL • *Institute of Genetics, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany*
FEDERICO TESSADORI • *Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands*
OLIVIER TURC • *Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, Montpellier, France*
COLIN G. N. TURNBULL • *Division of Biology, Imperial College London, London, UK*
JOACHIM F. UHRIG • *Botanical Institute III, University of Cologne, Köln, Germany*
ANDRÁS VICZIÁN • *Institute of Plant Biology, Hungarian Academy of Science, Biological Research Center, Szeged, Hungary*
CORINA B. R. VILLAR • *Department of Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland*
RONNY VÖLZ • *Center for Plant Molecular Biology (ZMBP), University of Tübingen, Tübingen, Germany*
GUY WACHSMAN • *Molecular Genetics Group, Department of Biology, Utrecht University, Utrecht, Netherlands*
SHAUL YALOVSKY • *Department of Plant Sciences, Tel-Aviv University, Tel-Aviv, Israel*

Chapter 1

Growth Protocols for Model Plants in Developmental Biology

Lars Hennig

Abstract

Arabidopsis is the dominating model species for plant developmental biology, but other species serve as models for processes that cannot be studied in *Arabidopsis*, such as compound leaf or wood formation, or to test the universality of developmental mechanisms initially identified in *Arabidopsis*. Research in plant developmental biology depends critically on robust growth protocols that will support reproducible development. Here, protocols are given to grow *Antirrhinum*, *Arabidopsis*, *Brachypodium*, maize, *Medicago*, *Petunia*, rice, and tomato in the laboratory.

Key words: *Antirrhinum*, *Arabidopsis*, *Brachypodium*, maize, *Medicago*, *Petunia*, rice, tomato.

1. Introduction

Research in plant developmental biology depends critically on robust growth protocols that will support reproducible development. Although *Arabidopsis thaliana* is the dominating model species for plant developmental biology, other species serve as models for processes that cannot be studied in *Arabidopsis*, such as compound leaf or wood formation, or to test the universality of developmental mechanisms initially identified in *Arabidopsis*.

A. thaliana is a member of the mustard family (*Brassicaceae*) with a broad natural distribution throughout Europe, Asia, and North America, and many accessions (ecotypes) can be obtained from stock centers. The most commonly used accessions are Columbia and Landsberg *erecta*. *Arabidopsis* has a life cycle of only 6 weeks (for a review about *Arabidopsis* as model species, *see* (1)).

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Snapdragon (*Antirrhinum majus)* is a member of the speedwell family (*Plantaginaceae*) and native to the Mediterranean. *A. majus* has a life cycle of 3–4 months. It has been used as a model for biochemical and developmental genetics for about a century, and many developmental regulatory genes were identified in *A. majus* by transposon tagging. *A. majus* is used to study processes such as the specification of flower and floral organ identity and leaf and flower asymmetry (for a review about *A. majus* as model species, *see* (2)).

Tomato (*Solanum lycopersicum*) is a domesticated member of the nightshade family (*Solanaceae*) and originated in western South America. Interesting developmental features of tomato include fleshy fruits, sympodial shoots, and compound leaves. Micro-Tom is an extremely small tomato variety (10–20 cm high), and due to its low-space requirements it is widely used for molecular research (3) (for a review about tomato as model species, *see* (4)).

Petunia is another member of the nightshade family originating from South America. *P. hybrida*, a hybrid of *P. axillaris* and *P. integrifolia*, is most commonly used in research. *P. hybrida* has a life cycle of only 8–12 weeks. A major attraction of *P. hybrida* is the presence of the extremely active endogenous dTph1 transposon system, which allows for efficient forward and reverse genetics (for reviews about *Petunia* as model species, *see* (5, 6)).

Barrel medic (*Medicago truncatula*) is a member of the pea family (*Fabaceae*) and native to the Mediterranean. It has a small diploid genome, is self-fertile, has a rapid generation time and prolific seed production and is also amenable to genetic transformation. Plants are 10- to 60-cm high. *M. truncatula* has a life cycle of 3–4 months. It serves mainly as model for nodulation and symbioses with nitrogen-fixing rhizobia, *Sinorhizobium meliloti,* and arbuscular mycorrhizal fungi (for a review about *M. truncatula* as model species, *see* (7)).

Rice (*Oryza sativa*) is a member of the grass family (*Poaceae*) and native to tropical and subtropical southern Asia. Plants grow 1–1.8 m tall. Rice has a life cycle of 4–6 months. It has a small diploid genome and is the most widely used model species for monocotyledonous plants (for a review about rice as model species, *see* (8)).

Maize (*Zea mays*) is another member of the grass family and was domesticated in Mesoamerica. Plants grow 2–3 m tall. Maize has a rich tradition in developmental genetics and large mutant collections exist (for a review about maize as model species, *see* (9)).

Purple false brome (*Brachypodium distachyon*), a third member of the grass family that is a model species for plant developmental biology, is native to southern Europe, northern Africa, and southwestern Asia. *B. distachyon* is a small (20-cm high), self-fertile, inbreeding annual weed. It has a life cycle of less than

4 months. *B. distachyon* has the simplest genome described in grasses to date, comparable to the *Arabidopsis* genome and several times smaller than the rice genome. *B. distachyon* is an emerging model for temperate grasses such as wheat (for a review about *B. distachyon* as model species, *see* (10)).

Detailed growth protocols for *Arabidopsis*, *Medicago*, *Antirrhinum*, and tomato can also be found elsewhere (11–13).

Poplar (*Populus trichocarpa*) is a member of the willow family (*Salicaceae*) and native to western North America. It is the preferred model to study perennial life cycles, bud dormancy, and wood formation (14). Because poplar has a long life cycle of several years, work with poplar differs substantially from raising annual or biannual herbaceous plants and will not be covered here. Models for nonflowering plants include the moss *Physcomitrella patents*, the green algae *Chlamydomonas reinhardtii*, and the fern *Ceratopteris richardii* and will also not be covered here.

2. Materials

2.1. Growing Arabidopsis

1. Gardening soil (*see* **Note 1**).
2. Pots (5–10 cm diameter).
3. Greenhouse or growth cabinet (22°C, 120–150 μmol m^{-2} s^{-1}) (*see* **Note 2**).

2.2. Growing Petunia

1. Gardening soil (*see* **Note 3**).
2. Pots (10–25 cm diameter).
3. Greenhouse (20–25° day, 18–20° night; >250 μmol m^{-2} s^{-1}, 16 h light).

2.3. Growing Tomato

1. Gardening soil (pH 6.0–6.8).
2. Pots (15 cm diameter).
3. Greenhouse or growth cabinet (18°C–24°C, 600–700 μmol m^{-2} s^{-1}).

2.4. Growing Antirrhinum

1. Gardening soil.
2. Pots (5–10 cm diameter).
3. Greenhouse (17°C–23°C; <70% humidity; >150 μmol m^{-2} s^{-1}; 16 h light).

2.5. Growing Brachypodium

1. Mix of gardening soil with vermiculite (2:1, v/v).
2. Pots (5–10 cm diameter).
3. Greenhouse or growth cabinet (24°C day, 18°C night; >150 μmol m^{-2} s^{-1}; 16 h light).

2.6. Growing Medicago

1. Mix of sand and gardening soil (1:2–1:3, v/v) (*see* **Note 4**).
2. Pots (8 and 20 cm diameter).
3. Greenhouse or growth cabinet (20–25°C day, 15–21°C night; 200–600 μmol m^{-2} s^{-1}; 16 h light).
4. Sand paper.

2.7. Growing Maize

1. Mix of sand and gardening soil (1:2–1:3, v/v).
2. Pots (10 and 25 cm diameter).
3. Greenhouse or growth cabinet (28°C day, 18°C night; >600 μmol m^{-2} s^{-1}; 16 h light).

2.8. Growing Rice

1. Growth medium: 1× MS salts (Sigma), 10% sucrose, 0.8% bacto agar, 100 mg/L inositol, 0.05 mg/L biotin, 0.5 mg/L pyridoxine HCl, 0.5 mg/L thiamin HCl, 5 mg/L nicotine acid, 0.5 mg/L folic acid, and 2 mg/L glycine (*see* **Note 5**).
2. Nutrient solution: 0.70 mM K_2SO_4, 0.10 mM KCl, 0.10 mM KH_2PO_4, 2.0 mM $Ca(NO_3)_2$, 0.50 mM $MgSO_4$, 10 μM H_3BO_3, 0.50 μM $MnSO_4$, 0.50 μM $ZnSO_4$, 0.20 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, and 100 μM Fe(III)-EDTA (pH 5.5) (*see* **Note 6**).
3. Mix of vermiculite and gardening soil (1:1, v/v).
4. Pots (18 cm diameter, 3 L).
5. Sterile transparent containers (5 cm diameter, 10 cm high).
6. Greenhouse or growth cabinet (28–32°C day, 20–25°C night; 80% humidity; >500 μmol m^{-2} s^{-1}; 12 h light).

3. Methods

3.1. Growing Arabidopsis

1. Fill pots with soil and compress very lightly to give a firm bed.
2. Sow the seeds onto the surface of the moist soil by scattering them carefully from a piece of folded cardboard; cover pots with a transparent lid to keep humidity high (*see* **Notes 7** and **8**).
3. Stratification at 4°C for 2–5 days improves germination rate and synchrony (*see* **Note 9**).
4. Transfer pots to growth cabinets or greenhouse, remove covers after 5–7 days, and then water pots from below so that pots can soak up water (*see* **Notes 10** and **11**).

5. Water plants until at least 90% of the seed pods have dried completely. Allow plants to dry slowly for maximum viable seed production.
6. After harvest, separate seeds from dry vegetative material and chaff using nylon mesh.
7. Seeds can be stored in paper bags in a dry atmosphere at room temperature for at least 3 years.

3.2. Growing Petunia

1. Fill pots with soil and compress very lightly to give a firm bed.
2. If desired, surface-sterilize seeds (1 min in 70% ethanol, 5 min in 2% bleach (sodium hypochlorite); rinse 5 times with sterile water).
3. Sow the seeds onto the surface of the moist soil.
4. Germination will take 3–7 days, flowering will occur after 10–12 weeks; seeds can be harvested after 13 weeks.
5. Harvest capsules and dry.
6. Store seeds at 4°C at low humidity.

3.3. Growing Tomato

1. Fill pots with soil and compress very lightly to give a firm bed.
2. Sow the seeds 3 cm apart in flats containing soil (0.5–2 cm from the surface of the soil) or wet filter paper. Cover with a plastic cover until seedlings emerge (7–14 days) to keep the substrate moist (*see* **Note 12**).
3. Place flats containing soil in a greenhouse or growth cabinet. Place flats with filter paper, until germination, in the dark at room temperature.
4. Transplant seedlings into pots.
5. Once flowering begins, shake the tomato plants gently once or twice each week to promote pollination.
6. Harvest ripe fruits (90–120 days after germination), cut in half with a knife, and squeeze the seeds into a container.
7. Mix seeds with excess tap water and incubate for ~3 d at room temperature to allow removal of the gelatinous seed coating.
8. Wash seeds extensively with tap water to remove the coating.
9. Dry seeds on a paper towel overnight at room temperature.

3.4. Growing Antirrhinum

1. Fill pots with soil and compress very lightly to give a firm bed.
2. Sow seeds on the surface of the soil and cover pots with a clear plastic cover to keep the soil moist (*see* **Note 13**).
3. Keep the pots at ~17°C.

4. After germination (7–10 days) (*see* **Note 14**), keep plants under high light at 17–23°C (*see* **Note 15**).
5. Water the plants as necessary (*see* **Note 16**).
6. Expect plants to flower 3–4 months after sowing.

3.5. Growing Brachypodium

1. Sow seeds into the soil.
2. Stratify seeds at 4°C for 1 week after sowing. To promote flowering by vernalization, extend incubation at 4°C to 3 weeks.
3. Transfer to growth cabinet or green house.

3.6. Growing Medicago

1. For mechanical scarification, place seeds on a fine-grade sand-paper sheet and rub them gently with another piece of sand paper until there are visible signs of abrasion (*see* **Note 17**).
2. Sow seeds on soil and cover with a fine dry sand/soil mixture (1:1, sieved to eliminate larger particles; 10–15 mm thick) for efficient rooting. Evenly spread and gently pack down this top layer using a flat tool (e.g., a Petri dish) and humidify it with a fine water spray. Cover with a clear plastic cover to maintain high humidity.
3. For dormant seeds, incubate for 48–72 h at 4°C.
4. If reduced growth and a shorter life cycle are desired, vernalize seedlings by incubating at 4°C for 10–14 days. Vernalized plants can flower as early as after 30 days (cultivar Jemalong), usually produce at least 15 pods (equivalent to 150 seeds), and can be grown at a density of up to 60 plants/m^2. Nonvernalized plants usually flower after 60–70 days, can yield several thousands of seeds per plant but need up to 1 m^2 per plant.
5. Incubate at 20°C in the dark until all viable seedlings have sprouted from the substrate (after 2–3 days).
6. Transfer into growth room, gradually remove (or puncture) the cover, and maintain watering with a fine spray for the first week.
7. Plant seedlings into small pots (8 cm). Once plants have at least 5 leaves (after ~2 weeks), transplant into large pots (20 cm) and transfer to glass house (*see* **Note 18**).
8. After pod harvest, allow pods to dry out at room temperature for ≥1 week at low humidity. Store dry, intact pods, at room temperature and low humidity, in strong paper envelopes or screw-cap plastic vials with punched holes in the cap to allow air circulation. Seeds normally retain good viability for at least 3–5 years under such conditions. Once removed from the fruits, seeds should be used within 1 month.

3.7. Growing Maize

1. If desired, surface-sterilize seeds with 6% hypochlorite for 10 min and wash 5 times with sterile water.
2. Plant seeds 1–3 cm deep into the soil in small pots (10 cm).
3. Place pots in growth room or greenhouse and water every 2–3 days with nutrient solution. Expect germination approximately 2–4 days after planting.
4. Transfer to large pots (25 cm) as required (usually after 15 days). Expect flowering after 6–12 weeks, depending on genotype.
5. Harvest seeds after 3–5 month.
6. Store seeds at 8°C at very low humidity.

3.8. Growing Rice

1. Surface-sterilize seeds: Shake in 70% ethanol for 3 min, shake in 6% sodium hypochlorite for 10 min, and wash four times with sterile water.
2. Place seeds on growth medium in sterile transparent containers and transfer to greenhouse.
3. Grow plantlets in the containers for about 2 weeks (until the leaves reach the lid of the container) and then transfer to soil or a hydroponics system. For transfer to soil, plantlets may be first grown in small pots (10 cm) and after another 2–3 weeks in large pots (18 cm) (*see* **Note 19**).
4. Plants will flower after about 4–5 months and first seeds can be harvested 4 weeks later (*see* **Note 20**).
5. Store seeds under dry conditions at 4°C.

4. Notes

1. Many commercially available soils work well. However, compost composition changes with the season and batch-to-batch variability can be high. Substrates based on peat are usually more consistent. Because water control is important, include a layer of perlite or vermiculite at the bottom of the pots to aid drainage. Alternatively, premixed soils that include vermiculite can be used.
2. Use continuous light photoperiods for fastest progression to flowering (after ~25 d). Long-day photoperiods (16 h of light) will result in slightly more vigorous plants (flowering after ~30 d). Use short-day photoperiods for prolonged vegetative development (flowering after ~60 d). Note that time estimates are for accession Columbia and will differ for other accessions.

3. Many commercially available soils work well. For mycorrhiza experiments, use a sand:soil mixture (2:1).
4. *M. truncatula* grows best on well-drained, fairly dense substrates such as mixtures of sand and soil. Alternatively, use mix of perlite and soil (1:3, v/v) or 100 % perlite, sand, or vermiculite. Perlite-grown plants are also ideal for nodulation assays. Wash perlite and vermiculite until pH is ~7.0 before use.
5. Less rich medium works well for germination as well. On the extreme side, seeds can be germinated on water-saturated Whatman paper.
6. Adjust the pH of the nutrient solution daily to 5.5 with 1 M HCl. Renew weekly.
7. Do not bury seeds in soil; *Arabidopsis* seeds require light for germination.
8. Seeds can be mixed with clean sand or glass beads for even distribution.
9. Vernalization is not required for common laboratory accessions, but exposure to 4°C for 6 weeks will accelerate flowering of many accessions from the wild.
10. One of the most common beginner's mistake is excess watering. Never allow excess water to remain in the tray.
11. *Arabidopsis* is predominantly self-fertilizing, but it is still advised to prevent direct contact between flowers from different lines. This can be easily achieved using plastic sleeves. Alternatively, plants can be fixed to stakes of metal wire or plastic rods.
12. Seeds of some accessions germinate poorly. To improve the germination rate, treat seeds in 2.7% bleach (sodium hypochlorite) for 30 min at room temperature and wash off the bleach completely by rinsing the seeds in water before sowing.
13. To facilitate sowing, seeds can be suspended in 0.1% agar and pipetted onto the soil.
14. Germination rate can be increased by imbibing seeds in 10 μM gibberellin solution for 3–5 d at 4°C before sowing.
15. Flowering is promoted by long days; a nighttime drop in temperature to 15–17°C increases apical meristem size and encourages robust stem growth.
16. To prevent wilting, it may be necessary to water plants twice daily. Increasing the size of pots and placing pots on capillary matting can reduce the need for watering. Avoid wetting the foliage to prevent fungal infections. All *Antirrhinum* species are intolerant to waterlogged soil. Avoid leaving pots in standing water.

17. The hydrophobic, waxy *M. truncatula* seed coat must be scarified in order to allow the penetration of water and oxygen that trigger germination.
18. Because *M. truncatula* is salt sensitive, water plants with deionised water instead of tap water and include complete fertilizer once every 1–2 weeks. Excessive watering will cause roots to rot with leaf wilting as the most common over-watering symptom. Therefore, do not over-water plants and allow the soil to partially dry out between watering.
19. Soil should have a low content of organic matter such as peat. Add 1 g of fertilizer (15% nitrogen, 10% phosphorus, 15% potassium, 2% magnesium, 0.05% boron, 0.1% copper, 0.05% iron, 0.1% manganese, 0.0001% cobalt, 0.0083% molybdenum, and 0.025% zinc) per 1 L of soil. Fill pots only 2/3 of their height with soil. Soil should be kept well watered at all times. Add water to the soil surface one to three times per day or place pots in tanks containing about 5–10 cm of room temperature water. Never use cold water for irrigation because roots are sensitive to low water temperature.
20. Most rice varieties are short-day plants that will flower sooner under 10-h than under 12-h photoperiods.

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References

1. Meinke, D. W., Cherry, J. M., Dean, C., Rounsley, S. D., and Koornneef, M. (1998) *Arabidopsis thaliana*: A model plant for genome analysis. *Science* 282, 679–682.
2. Schwarz-Sommer, Z., Davies, B., and Hudson, A. (2003) An everlasting pioneer: the story of *Antirrhinum* research. *Nat Rev Genet* 4, 657–666.
3. Meissner, R., Jacobson, Y., Melamed, S., Levyatuv, S., Shalev, G., Ashri, A., Elkind, Y., and Levy, A. (1997) A new model system for tomato genetics. *Plant J* 12, 1465–1472.
4. Kimura, S. and Sinha, N. (2008) Tomato (*Solanum lycopersicum*): A model fruit-bearing crop. *Cold Spring Harb Protoc*, doi:10.1101/pdb.emo105.