Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 32, 333-336, 2011

Plants—Don't Just Sit There, Do Something!!!

Marsha E. Fanning and Karen M. McDougal

Biology Program, Lenoir-Rhyne University, Hickory NC 28603 USA
(**fanning@lr.edu**; **mcdougalk@lr.edu**)

We've all done the basic plant labs where students look at flowers, seeds, and fruits. But how often have you actually shown students the "neat things" that are more hidden from view--the things they hear about but rarely see? In this workshop we show you how to prepare simple setups to view pollen tube germination, to see endosperm and early embryos of dicots, and to make preparations of mitosis in broad beans.

Keywords: Plants, botany, pollen tubes, chromosomes, embryo

Introduction

Some of the most fascinating botanical phenomena are rarely included in the introductory laboratory, and yet they are easily seen if the right plants are used. Three of these phenomena are included in this lab. All of the techniques have the potential for use in a variety of student exercises and research projects. Below are tried and true methods that students can use to view pollen tube germination, view large chromosomes, and watch embryo development.

© 2011 by Marsha E. Fanning and Karen M. McDougal

Student Outline

Part I. Pollen Tube Germination

- Add a few drops of germination medium to a microscope slide and add pollen grains to it by shaking a stamen over it or touching it to the liquid. (This process works best if there is not too much pollen.)
- Add a cover slip—not in the proper way, but by merely dropping it onto the liquid on the slide. This will trap air bubbles in the liquid, which seems to increase the percentage of pollen grains that germinate.
- Make note of the name of the plant from which your pollen came, and the time at which you added it to the pollination medium. (Note on slide with permanent marker.)
- Observe the pollen under the microscope, first under low, then under high power. Set the scope aside and return every 5 minutes to check for pollen tubes.

How long did it take for tubes to form? Can you see nuclei in them? (It may be necessary to adjust the light, focus and condenser settings to maximize detail.) If your pollen grains do not germinate, observe those of another student.

Part II. Fast plants: Endosperm and Early Embryos of Dicots

- From an approximately 22-day-old Fast Plant (*Brassica rapa*), remove a seedpod (fruit), place it on a glass slide and observe it with the dissecting microscope.
- Using two dissecting needles, or needles on tuberculin syringes, remove ovules from different-sized pods and place on the stage of the microscope in a small drop of water.
- Use the needles to pop open the ovule while looking through the microscope. Observe and draw several embryos, and determine their stage(s).
- Share your findings with others in the lab to observe as many stages as possible. For details of stages of ovular developments, see the Wisconsin Fast plant web page: **www.fastplants.org/pdf/activities/embryogenesis.pdf**.

Part III. Observing Mitosis in Broad Beans *(Vicia faba)* Root Tips

- Roots of 10-day-old Broad Beans have been harvested and stained using Schiff's reagent (which stains DNA but not RNA) and then placed in distilled water.
- Using fine forceps, pick up a single root and place it on a slide, then using another slide carefully "cut" off all but the stained tip (approximately the bottom 1-3 mm of the root). Add a drop of 45% acetic acid and a cover slip, being careful to prevent air bubbles.
- Tap the cover slip gently with a pencil eraser, until the "spot" is much paler and about 8-10 mm in diameter. Then place a folded Kimwipe on top of the cover slip, and press down firmly with your thumb.
- Observe the root squash under low power (10X). If the cells are in a monolayer (none or very few overlapping), continue with your observations. If cells are still stacked two or more deep, continue tapping with the pencil eraser until the squash is light pink and well spread, or lift the cover slip and add another drop of acetic acid and tap again.
- Locate some cuboidal cells under low power and then switch to high power. Look for actively dividing cells. Can you determine the number of chromosomes? What stages of mitosis are the cells in?

Notes for the Instructor

Part I. Pollen Tube Germination

Pollen grains may be stimulated to germinate by adding them to a pollination /germination medium which contains sugars (see recipe below).

Materials needed

Bee pollen
Pollen from freshly collected local flowers.

Germination medium
(Puterbaugh and Prince, 2004)

100 g sucrose (table sugar)
0.1 g boric acid (H_3BO_3)
0.3 g calcium nitrate ($Ca(N0_3)_2.4H_20$)
1 L distilled water

This works better if mixed as an entire liter, instead of in smaller quantities. It may be covered and refrigerated for a few days.

Part III. Observing Mitosis in Broad Beans (Vicia faba) Root Tips

- Plant Broad Beans in vermiculite in a plant tray ten days before needed for lab. Place under grow lights, and water as needed.
- The morning of lab, harvest roots. If possible this should be done around 9AM since mitosis is diurnal, with the greatest number of cells in various stages of mitosis in the morning.
- Remove a plant from the vermiculite, rinse it in water and use scissors to snip off a cm or two of all of the root tips into a small beaker of Farmer's solution (3 parts ethanol to 1 part glacial acetic acid). Leave in Farmer's for 2-3 hours.
- About 1 to 1 ½ hours before lab remove roots from Farmer's, rinse in distilled water (dip into small beaker of water) then put into 5N HCl for 30 minutes to hydrolyze the middle lamella and allow cells to separate.
- After 30 minutes in 5N HCl, rinse again in fresh distilled water, and place in Schiff's reagent for approximately 30 minutes to stain the nucleus. (Schiff's stains DNA but not RNA.) Remove root tips to a small beaker of distilled water after 30 minutes in order to prevent over-staining.

Alternate directions for preparing root tips ahead of time

- After roots have been collected and put into Farmer's solution for two to three hours, the roots can be rinsed in water, placed in glycerol, and frozen. When ready to use, rinse roots in distilled water 8 to 10 times, and then proceed as above with HCl and Schiff's reagent.

Recipe for Schiff's Reagent—Careful, this can get messy!

- Add 1 g basic Fuchsin to 200 mL boiling distilled water and boil for 1 minute while stirring. Be careful—this solution has a tendency to boil over!
- Cool to 50° C in an ice bath.
- Filter with aspirator using Buchner funnel and add 30 mL of 1 N HCl.
- Add 3 g of potassium metabisulfite (K_2SO_5) or sodium metabisulfite (Na_2SO_5). Cover with Parafilm and allow to bleach for 24 hrs in a dark place.
- After 24 hrs, add 1 g of decolorizing charcoal (carbon) and shake for 1 minute.
- Filter using aspirator and store in clean amber colored bottle. Label and store in refrigerator. Solution needs to be freshly made each semester.

Schiff's reagent can also be ordered from Carolina Biological Item #887265 Schiff's Reagent Laboratory Grade, 100 ml $13.25 US or Wards Item #947V8806 Schiff's Reagent Lab Solution 500 ml $19.90 US.

Literature Cited

Puterbaugh, Mary.. and Mary Blaine Prince. 2004. Using ant and butterfly pollination to involve students in scientific exploration. Pages 135-137, in *Tested Studies for Laboratory Teaching*, Volume 25 (M. A. O'Donnell, Editor). Proceedings of the 25th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 414 pages.

Wisconsin Fast Plants. 1998. Embryogenesis: Going for the globular. Wisconsin Fast Plants Program, University of Wisconsin- Madison [**www.fastplants.org/pdf/activities/embryogenesis.pdf**]

About the Authors

Marsha Fanning earned her doctorate in Zoology from the University of Georgia. She is a professor of biology in the Biology program at Lenoir-Rhyne University, where she teaches introductory biology courses as well as ecology, biodiversity and field biology.

Karen McDougal earned her doctorate in Botany from the University of North Carolina. She is a professor of biology in the Biology program at Lenoir-Rhyne University, where she teaches introductory biology courses as well as plant anatomy and physiology, systematic botany and environmental science.

Karen and Marsha have team taught numerous times, as well as collaborated in the supervision of numerous student research projects.

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/**

Papers published in *Tested Studies for Laboratory Teaching: Proceedings of the Conference of the Association for Biology Laboratory Education* are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

Although the laboratory exercises in this proceedings volume have been tested and due consideration has been given to safety, individuals performing these exercises must assume all responsibilities for risk. ABLE disclaims any liability with regards to safety in connection with the use of the exercises in this volume.

Citing This Article

Fanning, M.E., and McDougal, K.M. 2011. Plants– Don't Just Sit There Do Something!!! Pages 333-336, in *Tested Studies for Laboratory Teaching,* Volume 32 (K. McMahon, Editor). Proceedings of the 32nd Conference of the Association for Biol-ogy Laboratory Education (ABLE), 445 pages. **http://www.ableweb.org/volumes/vol-32/?art=31**

Compilation © 2011 by the Association for Biology Laboratory Education, ISBN 1-890444-14-6. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the copyright owner. Use solely at one's own institution with no intent for profit is excluded from the preceding copyright restriction, unless otherwise noted on the copyright notice of the individual chapter in this volume. Proper credit to this publication must be included in your laboratory outline for each use; a sample citation is given above. Upon obtaining permission or with the "sole use at one's own institution" exclusion, ABLE strongly encourages individuals to use the exercises in this proceedings volume in their teaching program.