Edward Chee Tak Yeung · Claudio Stasolla Michael John Sumner · Bing Quan Huang Editors

# Plant Microtechniques and Protocols



## Plant Microtechniques and Protocols

Edward Chee Tak Yeung • Claudio Stasolla Michael John Sumner • Bing Quan Huang Editors

### Plant Microtechniques and Protocols



**Editors** 

Edward Chee Tak Yeung

Department of Biological Sciences

University of Calgary

Calgary Alberta Canada

Claudio Stasolla

Department of Plant Sciences University of Manitoba

Winnipeg Manitoba Canada Michael John Sumner

Department of Biological Sciences

University of Manitoba

Winnipeg Manitoba Canada

Bing Quan Huang

Center for Basic Research in Digestive

Disease Mayo Clinic Rochester Minnesota USA

ISBN 978-3-319-19943-6 DOI 10.1007/978-3-319-19944-3 ISBN 978-3-319-19944-3 (eBook)

Library of Congress Control Number: 2015949269

Springer Cham Heidelberg New York Dordrecht London © Springer International Publishing Switzerland 2015

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Science+Business Media (www.springer.com)

#### **Preface**

Old and new techniques are essential for any research program. New instrumentation, techniques, methodologies, and ideas continue to appear in the literature. Some will have a profound influence on our future research activities. At present, many traditional methods are still routinely in use in many laboratories, and simple methods such as hand sections and related techniques are extremely useful in botanical research. It is unfortunate, however, that many traditional methods are not being taught. Students often lack a clear understanding of the methods used and, therefore, cannot take full advantage of the different microtechniques available for their experimental studies. The usefulness of many histological methods is that by providing basic structural information, they help in generating questions and hypotheses needed to advance research.

The purpose of putting together a volume related to plant microtechniques is to gather the commonly used methods and update their procedures using a simple and fully understandable approach. Although, many similar monographs have been published in the past, unfortunately, the majority of them are out of print. We hope this book can serve as a handy resource for scientists familiar with the protocols, and as a guide for the novices, especially students just beginning to learn about various structural methods for the first time.

In terms of organization, it is not possible to include all methods in a single volume. Many related techniques including those used for the study of animal biology, can be found in the Protocol Book Series. Readers are urged to look for specific methods by checking the "Protocol" website from Springer (www.springerprotocol.com). In the first section of this volume, we have selected the more commonly used embedding methods, with emphasis on the preparative methods for light and electron microscopy. A number of cell biology-related protocols are compiled in Sect. 2 to showcase the usefulness of various techniques based on different processing and staining methods. Section 3 highlights some common and recent procedures in wood preparation. The last section includes botanical methods related to archaeological uses of plant materials. A special chapter on field and herbarium procedures is also included to serve as a guide for students interested in plant collection and taxonomic studies. It is our aim to include a range of topics in order to generate cross

vi Preface

talks among scientists in different research disciplines. We realize that the methods selected are incomplete and hope to update and include new methods in the future.

We would like to thank Mr. Douglas Durnin for his careful proofreading of manuscripts and Mr. Colin Chan for his help in editing the figures and graphics. Finally, we are grateful to all authors for their contributions to this book and their patience and cooperation during the course of preparation and editing.

Edward Chee Tak Yeung Claudio Stasolla Michael John Sumner Bing Quan Huang

#### **Contents**

#### Part I Fixation, Processing, Embedding and Staining of Botanical Specimens

1	A Guide to the Study of Plant Structure with Emphasis on Living Specimens.  Edward C. Yeung	3
2	Chemical and Physical Fixation of Cells and Tissues: An Overview Bing Quan Huang and Edward C. Yeung	23
3	Paraffin and Polyester Waxes	45
4	The Glycol Methacrylate Embedding Resins—Technovit 7100 and 8100 Edward C. Yeung and Colin K. W. Chan	67
5	<b>Epoxy Resins for Light and Transmission Electron Microscopy</b> Michael John Sumner	83
6	LR White Acrylic Resin  Edward C. Yeung and Bing Quan Huang	103
7	High-Pressure Freezing and Freeze Substitution of In Vivo and In Vitro Cultured Plant Samples	117
8	Three-Dimensional Imaging for Electron Microscopy of Plastic-Embedded Plant Specimens Jose M. Seguí-Simarro	135

iii	Contents
-----	----------

9	Fluorescent Staining of Living Plant Cells	153
10	Improved Methods for Clearing and Staining of Plant Samples Alexander Lux, Marek Vaculík and Ján Kováč	167
Pa	rt II Microtechniques Related to Cell and Molecular Biology Studies	
11	Whole-Mount Immunofluorescence Staining of Plant Cells and Tissues  Masaki Shimamura	181
12	Protoplast Isolation and Staining	197
13	Guiding Principles for Live Cell Imaging of Plants Using Confocal Microscopy Subramanian Sankaranarayanan and Marcus A. Samuel	213
14	Immunogold Labeling for Electron Microscopy: Strategy and Problem Solving Fengli Guo and Bing Quan Huang	225
15	Abscisic Acid Immunostaining	251
16	Plant Chromosome Preparations and Staining for Light Microscopic Studies  Subhash C. Hiremath and C. C. Chinnappa	263
17	Chromosome Techniques and FISH	287
18	Detection of S-Phase of Cell Division Cycle in Plant Cells and Tissues by Using 5-Ethynyl-2'-Deoxyuridine (EdU)	311
19	Staining Methods for Programmed Cell Death  Owen S. D. Wally and Claudio Stasolla	323
20	Laser Microdissection of Plant Tissues  Jenna L. Millar, Michael G. Becker and Mark F. Belmonte	337

Contents ix

21	RNA In situ Hybridization	351
Pa	rt III Preparative Methods for the Study of Wood Anatomy	
22	Microscopic Examination of Wood: Sample Preparation and Techniques for Light Microscopy	373
23	Collecting and Processing Wood Microcores for Monitoring Xylogenesis  Annie Deslauriers, Sergio Rossi and Eryuan Liang	417
24	Three-Dimensional Imaging of Cambium and Secondary  Xylem Cells by Confocal Laser Scanning Microscopy  Satoshi Nakaba, Peter Kitin, Yusuke Yamagishi, Shahanara Begum,  Kayo Kudo, Widyanto Dwi Nugroho and Ryo Funada	431
Pa	rt IV Botanical Techniques and Protocols for Archeology and Herbarium Collection	
25	Collection of Plant Remains from Archaeological Contexts	469
26	Archaeological Wood Preparation  Alessandra Celant and Gemma Coccolini	487
27	Archaeopalynological Preparation Techniques  Donatella Magri and Federico Di Rita	495
28	<b>Phytoliths: Preparation and Archaeological Extraction</b>	507
29	<b>Starch Granules: Preparation and Archaeological Extraction</b> Brian Kooyman	525
30	Plant Collection, Identification, and Herbarium Procedures	541
Ind	day	573