

# Recent Advances in Polyphenol Research

Volume 4

*Edited by*  
Annalisa Romani,  
Vincenzo Lattanzio  
and Stéphane Quideau



WILEY Blackwell

# Table of Contents

[Recent Advances in Polyphenol Research](#)

[Title Page](#)

[Copyright](#)

[Dedication](#)

[Acknowledgments](#)

[Contributors](#)

[Preface](#)

[Chapter 1: Monolignol Biosynthesis and its Genetic Manipulation: The Good, the Bad, and the Ugly.](#)

[1.1 Introduction](#)

[1.2 Function and distribution of lignin in plants](#)

[1.3 Targets for modification of lignin biosynthesis](#)

[1.4 Impacts of lignin modification through targeting of the monolignol biosynthetic pathway.](#)

[1.5 Impacts of lignin modification through targeting of TFs](#)

[1.6 Monolignol pathway modification and plant growth](#)

[1.7 Conclusions: it isn't all that bad!](#)

[References](#)

[Chapter 2: Perturbing Lignin Biosynthesis: Metabolic Changes in Response to Manipulation of the Phenylpropanoid Pathway.](#)

[2.1 Introduction](#)

[2.2 Changes in metabolism associated with phenylpropanoid-pathway disruptions](#)

[2.3 Atypical lignins](#)

[2.4 Dwarfism](#)

[2.5 Conclusions](#)

[References](#)

[Chapter 3: Function, Structure, and Evolution of Flavonoid Glycosyltransferases in Plants](#)

[3.1 Introduction](#)

[3.2 UDP-dependent glycosyltransferases](#)

[3.3 Glycoside hydrolase-type glycosyltransferases](#)

[3.4 Conclusions](#)

[References](#)

[Chapter 4: The Chemistry and Chemical Ecology of Ellagitannins in Plant-Insect Interactions: From Underestimated Molecules to Bioactive Plant Constituents](#)

[4.1 Introduction](#)

[4.2 Definitions and chemical structures of hydrolyzable tannins](#)

[4.3 Biosynthetic pathways of hydrolyzable tannins in plants](#)

[4.4 Distributions of different types of tannin in plants](#)

[4.5 Tannins in plant-herbivore interactions](#)

[4.6 Conclusions](#)

[Acknowledgments](#)

[References](#)

[Chapter 5: Diverse Ecological Roles of Plant Tannins: Plant Defense and Beyond](#)

[5.1 Introduction](#)

[5.2 Overview of tannin structure and function in defense](#)

[5.3 Tissue localization and ecological function](#)

[5.4 Tannins in plant-soil-environment interactions](#)

[5.5 Conclusions](#)

[Acknowledgments](#)

[References](#)

[Chapter 6: Epigenetics, Plant \(Poly\)phenolics, and Cancer Prevention](#)

[6.1 Introduction](#)

[6.2 Influence of polyphenols on DNA methylation](#)

[6.3 Influence of polyphenols on histone-modifying enzymes](#)

[6.4 Influence of noncoding miRNAs on gene expression](#)

[6.5 Chemopreventive polyphenols affecting the epigenome via multiple mechanisms](#)

[6.6 Conclusions](#)

[References](#)

[Chapter 7: Discovery of Polyphenol-Based Drugs for Cancer Prevention and Treatment: The Tumor Proteasome as a Novel Target](#)

[7.1 Introduction](#)

[7.2 Secondary metabolites of plants](#)

[7.3 Plant polyphenols and their analogs](#)

[7.4 Natural polyphenols in reversal of drug resistance](#)

[7.5 Conclusions](#)

[Acknowledgments](#)

[References](#)

[Chapter 8: Flavonoid Occurrence, Bioavailability, Metabolism, and Protective Effects in Humans: Focus on Flavan-3-ols and Flavonols](#)

[8.1 Introduction](#)

[8.2 Focus on flavan-3-ols and flavonols: chemical structures and dietary sources](#)

[8.3 Metabolism and bioavailability of flavonoids in humans](#)

[8.4 \*In vitro\* studies](#)

[8.5 \*In vivo\* studies](#)

[8.6 Conclusions](#)

[References](#)

## [Chapter 9: Inhibition of VEGF Signaling by Polyphenols in Relation to Atherosclerosis and Cardiovascular Disease](#)

[9.1 Introduction](#)

[9.2 VEGF and VEGF signaling](#)

[9.3 VEGF signaling and angiogenesis](#)

[9.4 Angiogenesis and atherosclerosis](#)

[9.5 Polyphenols in foods and diets, and their absorption and metabolism](#)

[9.6 Effects of polyphenols on VEGF signaling, angiogenesis, and atherosclerosis](#)

[9.7 Relationships between polyphenol consumption and CVD risk](#)

[9.8 Conclusions](#)

[Acknowledgments](#)

[References](#)

## [Chapter 10: Phenolic Compounds from a Sex-Gender Perspective](#)

[10.1 Introduction](#)

[10.2 Phenolic compound classification and molecular mechanisms](#)

[10.3 Sex-gender and the xenokinetics of phenolic compounds](#)

[10.4 Sex-gender differences in xenodynamics](#)

[10.5 Conclusions](#)

[References](#)

[Chapter 11: Thermodynamic and Kinetic Processes of Anthocyanins and Related Compounds and their Bio-Inspired Applications](#)

[11.1 Introduction](#)

[11.2 Anthocyanins in aqueous solution](#)

[11.3 Influence of anthocyanin self-aggregation on the determination of rate and equilibrium constants](#)

[11.4 Photochromism: applications bio-inspired in anthocyanins](#)

[11.5 How to construct an energy-level diagram](#)

[11.6 How to calculate the mole-fraction distribution of a network species](#)

[References](#)

[Chapter 12: Synthetic Strategies and Tactics for Catechin and Related Polyphenols](#)

[12.1 Introduction](#)

[12.2 Early synthetic work](#)

[12.3 Stereoselective approaches to flavan-3-ols](#)

[12.4 Conclusions](#)

[Abbreviations](#)

[Acknowledgments](#)

[References](#)

[Index](#)

[End User License Agreement](#)

# List of Illustrations

[Figure P.1](#)

[Figure 1.1](#)

[Figure 1.2](#)

[Figure 1.3](#)

[Figure 1.4](#)

[Figure 2.1](#)

[Figure 2.2](#)

[Figure 3.1](#)

[Figure 3.2](#)

[Figure 3.3](#)

[Figure 3.4](#)

[Figure 3.5](#)

[Figure 4.1](#)

[Figure 4.2](#)

[Figure 4.3](#)

[Figure 4.4](#)

[Figure 4.5](#)

[Figure 4.6](#)

[Figure 4.7](#)

[Figure 4.8](#)

[Figure 4.9](#)

[Figure 4.10](#)

[Figure 4.11](#)

[Figure 4.12](#)

[Figure 4.13](#)

[Figure 4.14](#)

[Figure 4.15](#)

[Figure 4.16](#)

[Figure 5.1](#)

[Figure 5.2](#)

[Figure 6.1](#)

[Figure 6.2](#)

[Figure 6.3](#)

[Figure 6.4](#)

[Figure 7.1](#)

[Figure 7.2](#)

[Figure 8.1](#)

[Figure 8.2](#)

[Figure 8.3](#)

[Figure 9.1](#)

[Figure 9.2](#)

[Figure 9.3](#)

[Figure 9.4](#)

[Figure 11.1](#)

[Figure 11.2](#)

[Figure 11.3](#)

[Figure 11.4](#)

[Figure 11.5](#)

[Figure 11.6](#)



[Figure 11.7](#)

[Figure 11.8](#)

[Figure 11.9](#)

[Figure 11.10](#)

[Figure 11.11](#)

[Figure 11.12](#)

[Figure 11.13](#)

[Scheme 11.1](#)

[Scheme 11.2](#)

[Scheme 11.3](#)

[Scheme 11.4](#)

[Scheme 11.5](#)

[Scheme 11.6](#)

[Scheme 11.7](#)

[Scheme 11.8](#)

[Scheme 11.9](#)

[Scheme 11.10](#)

[Scheme 11.11](#)

[Scheme 11.12](#)

[Scheme 11.13](#)

[Figure 12.1](#)

[Figure 12.2](#)

[Figure 12.3](#)

[Figure 12.4](#)

[Figure 12.5](#)

[Figure 12.6](#)

[Figure 12.7](#)

[Figure 12.8](#)

[Figure 12.9](#)

[Figure 12.10](#)

[Figure 12.11](#)

[Figure 12.12](#)

[Figure 12.13](#)

[Figure 12.14](#)

[Figure 12.15](#)

[Figure 12.16](#)

[Figure 12.17](#)

[Figure 12.18](#)

[Figure 12.19](#)

[Figure 12.20](#)

[Figure 12.21](#)

[Figure 12.22](#)

[Figure 12.23](#)

[Figure 12.24](#)

[Figure 12.25](#)

[Figure 12.26](#)

[Figure 12.27](#)

[Figure 12.28](#)

[Figure 12.29](#)

[Figure 12.30](#)

[Figure 12.31](#)

## **List of Tables**

[Table 3.1](#)

[Table 3.2](#)

[Table 6.1](#)

[Table 6.2](#)

[Table 6.3](#)

[Table 6.4](#)

[Table 6.5](#)

[Table 6.6](#)

[Table 6.7](#)

[Table 6.8](#)

[Table 6.9](#)

[Table 6.10](#)

[Table 7.1](#)

[Table 7.2](#)

[Table 8.1](#)

[Table 8.2](#)

[Table 9.1](#)

[Table 9.2](#)

[Table 9.3](#)

[Table 9.4](#)

[Table 9.5](#)

[Table 11.1](#)

## **Recent Advances in Polyphenol Research**

A series for researchers and graduate students whose work is related to plant phenolics and polyphenols, as well as for individuals representing governments and industries with interest in this field. Each volume in this biennial series focuses on several important research topics in plant phenols and polyphenols, including chemistry, biosynthesis, metabolic engineering, ecology, physiology, food, nutrition, and health.

### **Volume 4 Editors:**

Annalisa Romani, Vincenzo Lattanzio, and Stéphane Quideau

### **Series Editor-in-Chief:**

Stéphane Quideau (University of Bordeaux, France)

### **Series Editorial Board:**

Véronique Cheynier (INRA, Montpellier, France)

Catherine Chéze (University of Bordeaux, France)

Gilles Comte (University of Lyon, France)

Fouad Daayf (University of Manitoba, Winnipeg, Canada)

Olivier Dangles (University of Avignon, France)

Kevin Davies (Plant & Food Research, Palmerston North, New Zealand)

Maria Teresa Escribano-Bailon (University of Salamanca, Spain)

Ann E. Hagerman (Miami University, Oxford, Ohio, USA)

Victor de Freitas (University of Porto, Portugal)

Johanna Lampe ((Fred Hutchinson Cancer Research Center, Seattle, Washington, USA)

Vincenzo Lattanzio (University of Foggia, Italy)

Virginie Leplanquais (LVMH Research, Christian Dior, France)

Stephan Martens (Fondazione Edmund Mach, IASMA, San Michele all'Adige, Italy)

Annalisa Romani (University of Florence, Italy)

Pascale Sarni-Manchado (INRA, Montpellier, France)

Celestino Santos-Buelga (University of Salamanca, Spain)

Kristiina Wähälä, (University of Helsinki, Finland)

Kumi Yoshida (University of Nagoya, Japan)

# Recent Advances in Polyphenol Research

## A Guide to SPSS, Data Analysis and Critical Appraisal

### Volume 4

Edited by

**Annalisa Romani**

*Professor, Food Science and Technology PHYTO LAB  
(Pharmaceutical, Cosmetic, Food supplement Technology  
and Analysis)*

*Department of Statistics, Informatics, Applications  
University of Florence, Italy*

**Vincenzo Lattanzio**

*Professor, Plant Biochemistry and Physiology  
Department of Sciences of Agriculture, Food and  
Environment*

*University of Foggia, Italy*

**Stéphane Quideau**

*Professor, Organic and Bioorganic Chemistry  
Institut des Sciences Moléculaires, CNRS-UMR 5255  
University of Bordeaux, France*

**WILEY Blackwell**

This edition first published 2014 © 2014 by John Wiley & Sons, Ltd

*Registered office:* John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

*Editorial offices:* 9600 Garsington Road, Oxford, OX4 2DQ, UK

The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at [www.wiley.com/wiley-blackwell](http://www.wiley.com/wiley-blackwell).

The right of the author to be identified as the author of this work has been asserted in accordance with the UK Copyright, Designs and Patents Act 1988.

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, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book.

**Limit of Liability/Disclaimer of Warranty:** While the publisher and author(s) have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. It is sold on the understanding that the publisher is not engaged in rendering professional services and neither the publisher nor the author shall be liable for damages arising herefrom. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

*Library of Congress Cataloging-in-Publication Data has been applied for*

ISBN 978-1-1183-2967-2

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Cover image: Image editing by Dr. Sandra Gallori, researcher at the Department of Neurosciences, Psychology, Drug Research and Child Health, Division of Pharmaceutical and Nutraceutical Sciences, University of Florence, Italy

## **Dedication**

This fourth volume of *Recent Advances in Polyphenol Research* is dedicated to the memory of Edwin Haslam, Professor of Physical-Organic Chemistry at the University of Sheffield, UK, who peacefully passed away at his home in Exeter on October 3, 2013, aged 81. Professor Haslam had been a long-standing and faithful member of Groupe Polyphénols, and was for many of us a model, a helpful mentor, a great colleague, and a friend. His pioneering and outstanding contributions to the field of plant polyphenols were, still are, and will continue to be a great source of knowledge and inspiration.



# Acknowledgments

The editors wish to thank all of the members of the Groupe Polyphénols Board Committee (2010–2012) for their guidance and assistance throughout this project.

## ***Groupe Polyphénols Board 2010-2012***

Dr. Véronique Cheynier

Dr. Catherine Chèze

Prof. Gilles Comte

Prof. Olivier Dangles

Dr. Kevin Davies

Prof. Victor de Freitas

Prof. Ann E. Hagerman

Dr. Johanna Lampe

Prof. Vincenzo Lattanzio

Dr. Virginie Leplanquais

Dr. Stephan Martens

Prof. Stéphane Quideau

Prof. Anna-Lisa Romani

Prof. Celestino Santos-Buelga

Prof. Kristiina Wähälä

Prof. Kumi Yoshida

## Contributors

**Nickolas A. Anderson**, Department of Biochemistry, Purdue University, West Lafayette, IN, USA

**Renato Bruni**, The  $\phi^2$  Laboratory of Phytochemicals in Physiology, LS9 Bioactives and Health Interlab Group, Department of Food Sciences, University of Parma, Parma, Italy

**Luca Calani**, The  $\phi^2$  Laboratory of Phytochemicals in Physiology, LS9 Bioactives and Health Interlab Group, Department of Food Sciences, University of Parma, Parma, Italy

**Ilaria Campesi**, Laboratory of Sex-Gender Medicine, National Institute of Biostructures and Biosystems, Osilo, Italy

**Tak Hang Chan**, Department of Chemistry, McGill University, Montreal, QC, Canada

**Clint Chapple**, Department of Biochemistry, Purdue University, West Lafayette, IN, USA

**Di Chen**, Department of Oncology and Barbara Ann Karmanos Cancer Institute, School of Medicine, Wayne State University, Detroit, MI, USA

**C. Peter Constabel**, Centre for Forest Biology, Department of Biology, University of Victoria, Victoria, BC, Canada

**Margherita Dall'Asta**, The  $\phi^2$  Laboratory of Phytochemicals in Physiology, LS9 Bioactives and Health Interlab Group, Department of Food Sciences, University of Parma, Parma, Italy

**Daniele Del Rio**, The  $\phi^2$  Laboratory of Phytochemicals in Physiology, LS9 Bioactives and Health Interlab Group, Department of Food Sciences, University of Parma, Parma, Italy

**Richard A. Dixon**, Department of Biological Sciences, University of North Texas, Denton, TX, USA

**Q. Ping Dou**, Departments of Oncology, Pharmacology, and Pathology and Barbara Ann Karmanos Cancer Institute, School of Medicine, Wayne State University, Detroit, MI, USA

**Rebecca L. Edwards**, Institute of Food Research, Norwich Research Park, Norwich, UK

**Flavia Franconi**, Department of Biomedical Science, Centre of Excellence for Biotechnology Development and Biodiversity, University of Sassari, Sassari, Italy

**Lina Gallego-Giraldo**, Department of Biological Sciences, University of North Texas, Denton, TX, USA

**Clarissa Gerhauser**, Epigenomics and Cancer Risk Factors, German Cancer Research Center (DKFZ), Heidelberg, Germany

**Fathima R. Kona**, Department of Oncology and Barbara Ann Karmanos Cancer Institute, School of Medicine, Wayne State University, Detroit, MI, USA

**Paul A Kroon**, Polyphenols and Health Group, Institute of Food Research, Norwich Research Park, Norwich, UK

**Maria Marino**, Department of Biology, University Roma Tre, Rome, Italy

**Ken Ohmori**, Department of Chemistry, Tokyo Institute of Technology, Tokyo, Japan

**Fernando Pina**, REQUIMTE, Department of Chemistry, Faculty of Science and Technology, New University of

Lisbon, Lisbon, Portugal

**M. S. Srinivasa Reddy**, Forage Genetics International,  
West Salem, WI, USA

**Annalisa Romani**, PHYTOLAB, Department of  
Statistics, Informatics, Applications, University of  
Florence, Florence, Italy

**Kazuki Saito**, RIKEN Center for Sustainable Resource  
Science, Yokohama, Japan, and Graduate School of  
Pharmaceutical Sciences, Chiba University, Chiba, Japan

**Juha-Pekka Salminen**, Laboratory of Organic  
Chemistry and Chemical Biology, Department of  
Chemistry, University of Turku, Turku, Finland

**Min Shen**, Department of Pharmacology, Department of  
Oncology and Barbara Ann Karmanos Cancer Institute,  
School of Medicine, Wayne State University, Detroit, MI,  
USA

**Keisuke Suzuki**, Department of Chemistry, Tokyo  
Institute of Technology, Tokyo, Japan

**Vincent Walker**, Centre for Forest Biology, Department  
of Biology, University of Victoria, Victoria, BC, Canada

**Keiko Yonekura-Sakakibara**, RIKEN Center for  
Sustainable Resource Science, Yokohama, Japan

**Kazuko Yoshida**, Centre for Forest Biology, Department  
of Biology, University of Victoria, Victoria, BC, Canada

# Preface

During the last 10 years there has been increasing interest in the study of plant polyphenols and their innumerable roles in a variety of very different contexts. Plant polyphenols are secondary metabolites and constitute one of the most common and widespread groups of substances in plants. Their structural diversity is likely the result of plant adaptive responses to natural selection.

Polyphenols express a large and diverse range of beneficial effects in plants and in humans consuming plant-derived food and beverages. For example, polyphenols are well known for their antioxidation activity, hormone-like behavior, and role as natural neurotransmitters, among many other biological activities. They also provide antimicrobial activity for the plant's own defense against invading pathogens.

The diversity of structures and activities of plant polyphenolic compounds has resulted in the emergence of numerous investigations in various and often interdisciplinary research areas, encompassing scientific domains as diverse as chemistry, biochemistry, biotechnology, ecology, physiology, nutrition and food chemistry, pharmacy and medicine, cosmetics, and textile technology, as well as in quality and environment controls and assessments.

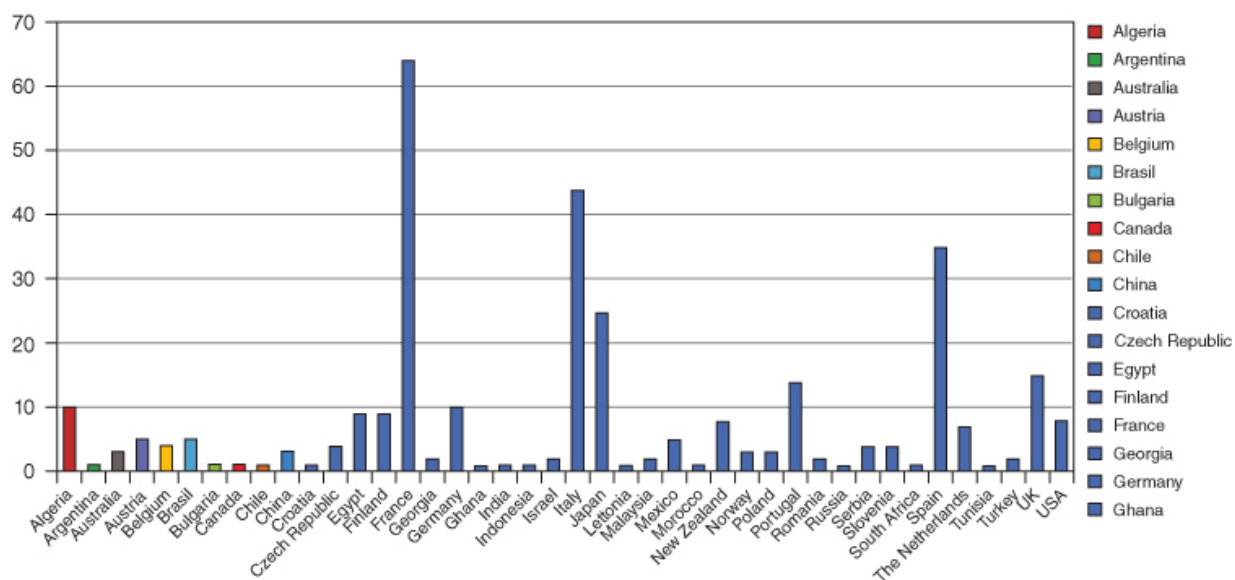
It is thus the aim of the International Conference on Polyphenols, which is a biennial event that is organized under the auspices of Groupe Polyphénols, to provide scientists across disciplines with a forum for sharing new findings and for exchanging views and ideas on polyphenol research at large.

For the first time in its history, in 2012 the 26th International Conference on Polyphenols was organized in Florence, Italy. The interest in polyphenol science at the University of Florence involves many departments, including Pharmaceutical Sciences, Chemistry, Plant Sciences and Ecology, Food Science, and Medicine, as well as The Multidisciplinary Centre of Research on Food Sciences (CeRA - MCRFS) and the laboratory of Commodity Sciences and Quality Control, Environment Assessments and Certification. In these fields, particular attention has been dedicated to functional-food, nutraceutical, and cosmeceutical discoveries and applications.

At the 26th International Conference on Polyphenols, five different main topics were selected for the scientific program:

1. *Phenols and Polyphenols Chemistry*: Covering (i) isolation and structural elucidation, and (ii) synthesis, reactivity, and physical-chemical properties.
2. *Biosynthesis, Genetics, and Metabolic Engineering*: Dealing with biosynthesis and genetic manipulation.
3. *Roles in Plants and Ecosystems*: Covering phenolic functions in plants and correlation with biotic and abiotic stresses.
4. *Health and Nutrition*: Focusing on polyphenol metabolism and bioavailability, as well as cancer prevention and perspectives on gender-dependent human health effects.
5. *Polyphenols and Drug Discovery*: Including new findings on sources of isolated and standardized polyphenolic fractions and novel epigenetic polyphenol mechanisms.

More than 400 scientists from 42 countries attended the conference in July 2012, with nearly 400 paper contributions, comprising 52 oral communications and 327 poster presentations ([Fig. P.1](#)).



**Fig. P.1** Contributions to the 26th International Conference on Polyphenols (number of papers presented) by country.

The success of this 26th edition of the International Conference on Polyphenols would not have been possible without the support of both public and private sponsors. The Scientific and Technological Pole and the Social Pole of the University of Florence, PIN of Prato, the National Council for Research, and several private-company sponsors (Agilent Technologies, BioTech Power, Indena, ISR Ecoindustria, Domus Olea, Force A, Biokyma, PhenoFarm, Dermaresia, Silva Team, Bioscen Future) are gratefully acknowledged.

All of the lectures, oral communications, and ensuing discussions and debates were broadcast live on RadioSpin, the University of Florence webradio, and through Ustation (the Italian university radio stations network), on the other connected university radios of the network. These

radiophonic conference proceedings are available in podcasts on the RadioSpin Web site: [www.radiospin.it](http://www.radiospin.it).

Annalisa Romani  
Vincenzo Lattanzio  
Stéphane Quideau



# Chapter 1

## Monolignol Biosynthesis and its Genetic Manipulation: The Good, the Bad, and the Ugly

Richard A. Dixon<sup>1</sup>, M.S. Srinivasa Reddy<sup>2</sup>, and Lina Gallego-Giraldo<sup>1</sup>

<sup>1</sup>*Department of Biological Sciences, University of North Texas, Denton, TX, USA*

<sup>2</sup>*Forage Genetics International, West Salem, WI, USA*

**Abstract:** Economic and environmental factors favor the adoption of lignocellulosic bioenergy crops for production of liquid transportation fuels. However, lignocellulosic biomass is recalcitrant to saccharification (sugar release from cell walls), and this is, at least in part, due to the presence of the phenylpropanoid-derived cell-wall polymer lignin. A large body of evidence exists documenting the impacts of lignin modification in plants. This technology can lead to improved forage quality and enhanced processing properties for trees (paper pulping) and lignocellulosic energy crops. We here provide a comprehensive review of the literature on lignin modification in plants. The pathway has been targeted through down-regulation of the expression of the enzymes of the monolignol pathway and down-regulation or over-expression of the transcription factors that control lignin biosynthesis and/or programs of secondary cell-wall development. Targeting lignin modification at some steps in the monolignol pathway can result in impairment of plant growth and development, often associated with the triggering of endogenous host-defense mechanisms. Recent

studies suggest that it may be possible to decouple negative growth impacts from lignin reduction.

**Keywords:** monolignol biosynthesis, genetic modification, transcription factor, gene silencing, saccharification

## 1.1 Introduction

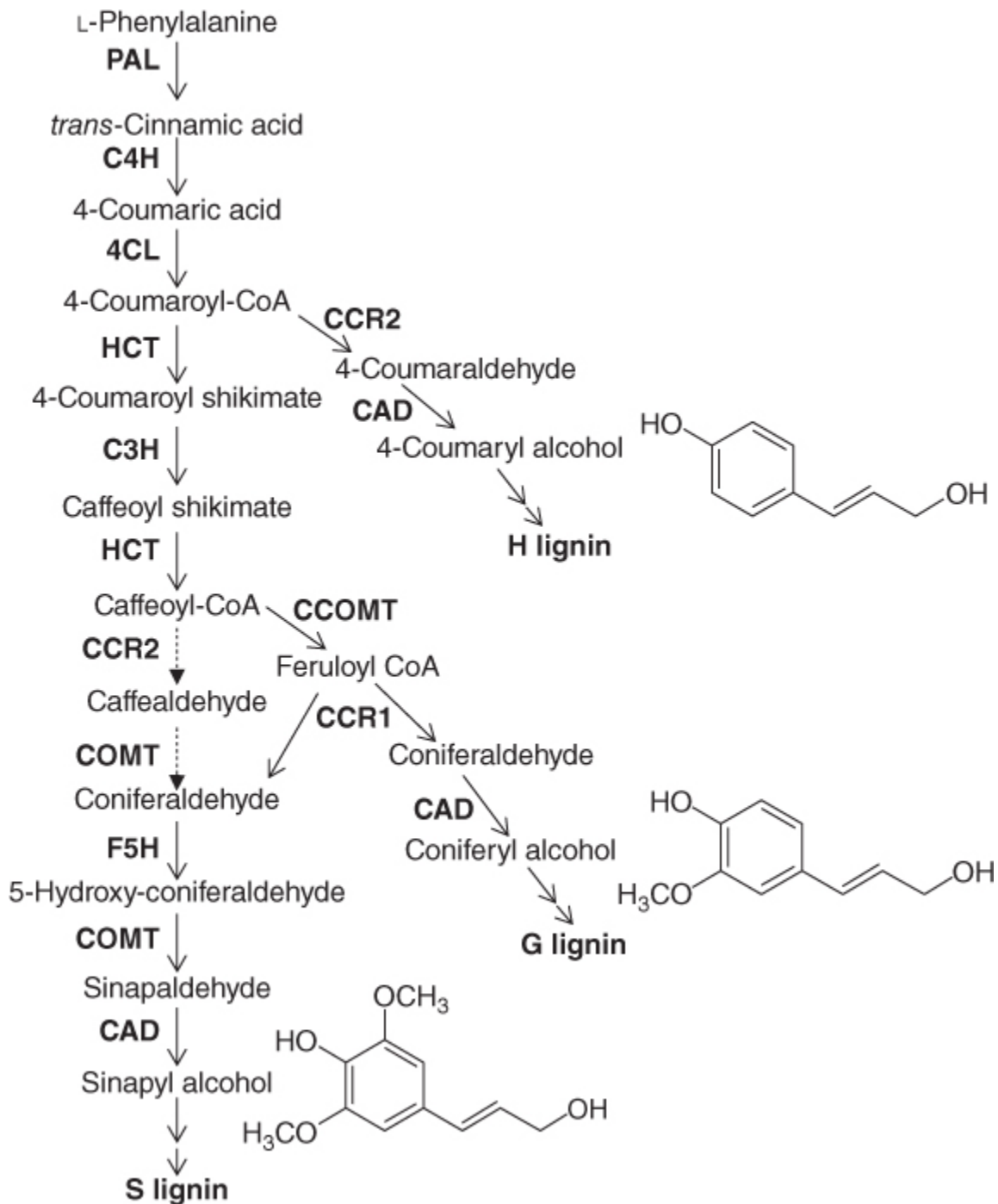
Lignin is a major component of plant secondary cell walls, and the second most abundant plant polymer on the planet. It constitutes about 15–35% of the dry mass of vascular plants (Adler, 1977). Considerable attention has been given over the past several years to the reduction of lignin content in model plant species, forages, trees, and dedicated bioenergy feedstocks. This is because forage digestibility, paper pulping, and liquid fuel production from biomass through fermentation are all affected by recalcitrance of lignocellulose, primarily due to the presence of lignin, which blocks access to the sugar-rich cell-wall polysaccharides cellulose and hemicellulose for enzymes and microorganisms (Pilate *et al.*, 2002; Reddy *et al.*, 2005; Chen & Dixon, 2007).

Much is now known of the biosynthesis of lignin and its control at the transcriptional level. This informs the targets that have been selected for genetic modification of lignin content and composition in transgenic plants. Which gene is down- or up-regulated has a considerable effect on lignin content and composition. Equally, lignin modification can have profound impacts on plant growth and development, ranging from good through bad to “downright ugly,” but these impacts are again strongly target-dependent. Understanding the mechanisms that can impact plant growth—which equate to agronomic performance—in crop species “improved” through lignin modification is critical for economic advancement of the forage and biofuels industries. Although still poorly understood, these

mechanisms may also throw light on basic plant developmental and defense processes.

## 1.2 Function and distribution of lignin in plants

Lignin is an aromatic heteropolymer derived primarily from three hydroxycinnamyl alcohols: 4-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which give rise, respectively, to the 4-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) subunits of lignin (Freudenberg & Neish, 1968; Ralph *et al.*, 2004). G units are mono-methoxylated, S units are di-methoxylated, and H units are not methoxylated ([Fig. 1.1](#)). These monomers are linked through oxidative coupling catalyzed by both peroxidases and laccases (Boudet *et al.*, 1995). Unlike cellulose and other polymers that have labile linkages (e.g. glycosidic or peptide) between their building blocks, the units of lignin are linked by strong ether and carbon-carbon bonds (Sarkanen, 1971). Lignin is present in the secondarily thickened cell walls of plants, where it is critical to cell-wall structural integrity and gives strength to stems (Chabannes *et al.*, 2001b; Jones *et al.*, 2001). Lignin also imparts hydrophobicity to vascular elements for water transport. The lignin content of the mature internodes of stems of alfalfa (*Medicago sativa*), the world's major forage legume and a target of much of the work to be described in this article, is about 17% of the dry weight (Guo *et al.*, 2001a).



**Fig. 1.1** Scheme for monolignol biosynthesis in dicotyledonous angiosperms, including revisions encompassing the different biochemical activities of cinnamoyl-CoA reductase (CCR) forms in *Medicago truncatula* (Zhou *et al.*, 2010). See text for enzyme abbreviations.

Lignin composition varies among major phyla of vascular plants (Boerjan *et al.*, 2003). Dicotyledonous and monocotyledonous angiosperm lignins contain G and S units as the two major monomer species, with low levels of H units. Monocotyledonous lignins have more H units than dicotyledonous lignins (Baucher *et al.*, 1998), but care must be taken not to attribute other components to H units, as often happens (Boerjan *et al.*, 2003). Fern and gymnosperm lignins have primarily G units and low levels of H units, but S units have been found in cuplet fern, yew plum pine, sandarac-cypress, and a few genera in the Gnetophyta (Weng *et al.*, 2008b). Some lower plants, like *Selaginella moellendorffii* (Weng *et al.*, 2008a,b) and *Marchantia polymorpha*, have both G and S units in their lignins (Espineira *et al.*, 2011), despite predating hardwoods/dicots and even softwoods. The apparent presence of H, G, and S units in the lignin from the seaweed *Calliarthron cheilosporioides* (Martone *et al.*, 2009) may indicate convergent evolution of lignin.

The presence of each methoxyl group on a monolignol unit results in one less reactive site, and therefore fewer available potential coupling combinations during polymerization. Thus, S lignin is more linear and less crosslinked than G/S lignin, and provides a strong yet flexible polymer that is especially advantageous to herbaceous angiosperms (Bonavitz & Chapple, 2010). A correlation has been shown between the degradability of the cell walls in forages and the amount of G lignin, as lignin rich in G units is more highly condensed, making it less amenable to degradation (Jung & Deetz, 1993). Thus, transgenic poplar plants with lignin rich in G units are, like softwoods, more difficult to pulp because of their more condensed lignin (Lapierre *et al.*, 1999).

Lignin content increases with progressive maturity of stems; this relationship has been studied in detail in alfalfa

(Jung *et al.*, 1997; Chen *et al.*, 2006), ryegrass (Tu *et al.*, 2010), tall fescue (Buxton & Redfearn, 1997; Chen *et al.*, 2002), and switchgrass (Mann *et al.*, 2009; Shen *et al.*, 2009). Decreasing the lignin content increases the digestibility of alfalfa for ruminant animals (Baucher *et al.*, 1999; Guo *et al.*, 2001a,b; Reddy *et al.*, 2005) and improves processing efficiency for the production of liquid biofuels through saccharification and fermentation (Chen & Dixon, 2007). Lignin composition has also been linked with reduced cell-wall digestibility (Jung & Deetz, 1993). However, the importance of lignin composition for digestibility has been questioned based on the results of studies with synthetic lignins, which show lignin composition *per se* to have no effect (Grabber *et al.*, 1997).

Plants have primary and secondary cell walls, which differ in both function and composition. Primary walls allow cells to expand and divide, while providing mechanical strength. Once cell growth stops, a much thicker secondary cell wall is deposited in some specialized cell types. These include vessels and fibers in the stem, sclereid cells, endodermal tissue of roots, some cells of anthers and pods important for dehiscence (Zhong & Ye, 2009), and seed coats (Marles *et al.*, 2008; Chen *et al.*, 2012). Generally, secondary cell walls consist of three layers, named S1 (outer), S2 (middle), and S3 (inner). Lignin deposition starts at the cell corners in the region of the middle lamella and the primary wall when S1 formation has started. Most of the lignin is deposited in the S2 layer and impregnates the cellulose and hemicelluloses there (Donaldson, 2001; Boerjan *et al.*, 2003). Based on UV microscopy, the density of lignin is higher in the middle lamella and primary walls than in the secondary walls of secondarily thickened cells, but the secondary walls have more lignin content as they constitute the largest proportion of the total cell wall (Fergus *et al.*, 1969). Usually H units are deposited first during cell-wall