Nitric Oxide in Plant Physiology

Edited by
Shamsul Hayat, Masaki Mori, John Pichtel, and Aqil Ahmad
Related Titles

Hirt, H. (ed.)

**Plant Stress Biology**
*From Genomics to Systems Biology*
2010
ISBN: 978-3-527-32290-9

Kahl, G., Meksem, K. (eds.)

**The Handbook of Plant Functional Genomics**
*Concepts and Protocols*
2008
ISBN: 978-3-527-31885-8

Ahmad, A., Pichtel, J., Hayat, S. (eds.)

**Plant-Bacteria Interactions**
*Strategies and Techniques to Promote Plant Growth*
2008
ISBN: 978-3-527-31901-5

Scott, P.

**Physiology and Behaviour of Plants**
2008
ISBN: 978-0-470-85025-1

Buchanan, B., Gruissem, W., Jones, R. L. (eds.)

**Biochemistry & Molecular Biology of Plants**
2002
Nitric Oxide in Plant Physiology

Edited by
Shamsul Hayat, Masaki Mori, John Pichtel,
and Aqil Ahmad

WILEY-VCH Verlag GmbH & Co. KGaA
The Editors

Dr. Shamsul Hayat
Aligarh Muslim University
Department of Botany
Aligarh 202002
India

Dr. Masaki Mori
National Institute of Agrobiological Sciences
Division of Plant Sciences
2-1-2 Kannondai, Tsukuba
Ibaraki 305-8602
Japan

Prof. John Pichtel
Ball State University
Department of Natural Resources and
Environmental Management
WQ 103
Muncie, IN 47306
USA

Dr. Aqil Ahmad
Higher College of Technology
Department of Applied Sciences
Al-Khuwair
Sultanate of Oman
and
Aligarh Muslim University
Department of Botany
Aligarh 202002
India

All books published by Wiley-VCH are carefully produced. Nevertheless, authors, editors, and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at http://dnb.d-nb.de.

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Cover  Formgeber, Eppelheim
Typesetting  Thomson Digital, Noida, India
Printing and Binding  Strauss GmbH, Mörlenbach

Printed in the Federal Republic of Germany
Printed on acid-free paper

ISBN: 978-3-527-32519-1
Contents

Preface XI
List of Contributors XIII

1 Nitric Oxide: Chemistry, Biosynthesis, and Physiological Role 1
Shamsul Hayat, Syed Aiman Hasan, Masaki Mori, Qazi Fariduddin, and Aqil Ahmad
1.1 Introduction 1
1.2 Nitric Oxide Chemistry 2
1.3 Biosynthesis of Nitric Oxide 3
1.4 Physiological Role of Nitric Oxide 5
1.4.1 Effect of Nitric Oxide on Seed Dormancy 5
1.4.2 Effect of Nitric Oxide on Growth 6
1.4.3 Effect of Nitric Oxide on Senescence 6
1.4.4 Effect of Nitric Oxide on Nitrate Reductase Activity 7
1.4.5 Effect of Nitric Oxide on Respiration 7
1.4.6 Effect of Nitric Oxide on Stomatal Movement 7
1.4.7 Effect of Nitric Oxide on Chlorophyll Content 7
1.4.8 Effect of Nitric Oxide on Photosynthesis 8
1.4.9 Effect of Nitric Oxide on Antioxidant System 8
1.4.10 Effect of Nitric Oxide on Programmed Cell Death 9
1.5 Nitric Oxide and Cross Talk with Classical Plant Hormones 10
1.5.1 Auxins and Nitric Oxide 10
1.5.2 Abscisic Acid and Nitric Oxide 11
1.5.3 Cytokinins, Gibberellins, and Nitric Oxide 11
1.5.4 Ethylene and Nitric Oxide 12
References 12

2 Electron Paramagnetic Resonance as a Tool to Study Nitric Oxide Generation in Plants 17
Susana Puntarulo, Sebastián Jasid, Alejandro D. Boveris, and Marcela Simontacchi
2.1 Introduction 17
2.1.1 Chemistry of Nitrogen-Active Species 17
2.1.2 Biological Effects of NO 18
2.2 Methods of NO Detection 19
2.2.1 Determination of NO by Specific Electrodes 19
2.2.2 Determination of NO by Spectrophotometric and Fluorometric Methods 19
2.2.3 Determination of NO by Electron Paramagnetic Resonance 20
2.2.3.1 Specific Experimental Advances 20
2.3 Use of EPR Methodology for Assaying Enzyme Activities 22
2.3.1 NOS-Like Dependent NO Generation 24
2.3.2 Nitrate Reductase-Dependent NO Generation 24
2.4 Application of EPR Methods to Assess NO Generation During Plant Development 26
2.5 Conclusions 27
References 27

3 Calcium, NO, and cGMP Signaling in Plant Cell Polarity 31
Ana Margarida Prado, José A. Feijó, and David Marshall Porterfield
3.1 Introduction 31
3.2 Cell Polarity and Plant Gametophyte Development 33
3.3 Calcium Signaling in Pollen and Fern Spores 34
3.4 NO/cGMP Signaling in Pollen and Fern Spores 35
3.5 NO/cGMP in Pollen–Pistil Interactions 38
3.6 Ovule Targeting and NO/cGMP 39
3.7 Ca^{2+}/NO/cGMP Connection? 42
3.8 Closing Perspectives 46
References 48

4 Nitric Oxide and Abiotic Stress in Higher Plants 51
Francisco J. Corpas, José M. Palma, Marina Leterrier, Luis A. del Río, and Juan B. Barroso
4.1 Introduction 51
4.2 Nitric Oxide and Related Molecules 52
4.2.1 Chemistry of Nitric Oxide in Plant Cells 52
4.2.2 Reactive Nitrogen Species 52
4.3 Cellular Targets of NO 54
4.3.1 Nitrosylated Metals 54
4.3.2 Protein S-Nitrosylation 55
4.3.3 Protein Tyrosine Nitration 55
4.3.4 Nitrolipids 55
4.3.5 Nucleic Acid Nitration 56
4.3.6 NO and Gene Regulation 56
4.4 Functions of NO in Plant Abiotic Stress 57
4.4.1 Salinity 57
4.4.2 Ultraviolet Radiation 58
4.4.3 Ozone 58
4.4.4 Mechanical Wounding 59
4.4.5 Toxic Metals (Cadmium and Aluminum) 59
4.5 Concluding Remarks 60
References 61

5 Polyamines and Cytokin: Is Nitric Oxide Biosynthesis the
Key to Overlapping Functions? 65
Rinukshi Wimalasekera and Günther F.E. Scherer
5.1 Introduction 65
5.2 Cytokinin- and Polyamine-Induced NO Biosynthesis 66
5.3 Tissue Distribution of Zeatin-Induced and PA-Induced
NO Formation 67
5.4 Nitric Oxide, Cytokininin, and Polyamines in Plant Growth and
Development and in Abiotic and Biotic Stresses 68
5.4.1 Embryogenesis 68
5.4.2 Flowering 69
5.4.3 Senescence 69
5.4.4 Programmed Cell Death 69
5.4.5 Abiotic Stresses 70
5.4.6 Biotic Stresses 71
References 73

6 Role of Nitric Oxide in Programmed Cell Death 77
Michela Zottini, Alex Costa, Roberto De Michele, and Fiorella Lo Schiavo
6.1 Programmed Cell Death in Plants 77
6.1.1 PCD Hallmarks and Regulation 78
6.2 NO as a Signaling Molecule 79
6.2.1 NO Is Able to Induce or Inhibit PCD 79
6.2.2 Nitric Oxide and PCD in Hypersensitive Response 80
6.2.3 Signaling Component in SA-Induced NO Production 80
6.3 Role of Mitochondria in NO-Induced PCD 84
6.4 Conclusions 85
References 85

7 Nitrate Reductase-Deficient Plants: A Model to Study Nitric
Oxide Production and Signaling in Plant Defense Response
to Pathogen Attack 89
Ione Salgado, Halley Caixeta de Oliveira, and Marcia Regina Braga
7.1 Introduction 89
7.2 Physicochemical Basis of NO Signaling 91
7.3 Defense Responses Mediated by NO 92
7.3.1 Accumulation of Defensive Compounds 92
7.3.2 Hypersensitive Response 93
7.3.3 Systemic Responses 94
7.3.4 Stomatal Closure 94
7.4 Substrates for NO Production During Plant–Pathogen Interactions 95
7.4.1 Production of NO from l-Arginine 95
7.4.2 Production of NO from Nitrite 95
7.5 The Role of Nitrate Reductase in NO Production During Plant–Pathogen Interactions 97
References 98

8 Effective Plant Protection Weapons Against Pathogens Require “NO Bullets” 103
Luzia V. Modolo

8.1 Introduction 103
8.2 Nitric Oxide and Reactive Oxygen Species in the Hypersensitive Response 104
8.3 Nitric Oxide and Phytoalexin Production 107
8.4 Nitric Oxide and the Salicylic Acid Signaling Pathway 108
8.5 Nitric Oxide and the Jasmonic Acid Signaling Pathway 109
8.6 Nitric Oxide and Gene Regulation 109
8.7 Nitric Oxide and Protein Regulation 110
8.8 Concluding Remarks 111
References 111

9 The Role of Nitric Oxide as a Bioactive Signaling Molecule in Plants Under Abiotic Stress 115
Gang-Ping Hao and Jian-Hua Zhang

9.1 Introduction 116
9.2 Biosynthesis of Nitric Oxide Under Abiotic Stress 116
9.2.1 NO Generated from NOS-Like Activity Under Abiotic Stress 116
9.2.2 NO Generated from NR Under Abiotic Stress 120
9.3 NO Signaling Functions in Abiotic Stress Responses 121
9.3.1 Function of NO Under Drought Stress 122
9.3.2 Function of NO Under Salt Stress 123
9.3.3 Function of NO Under Ultraviolet Radiation 125
9.3.4 Function of NO Under Heat and Low Temperature 126
9.3.5 Function of NO Under Heavy Metal Stress 126
9.3.6 Function of NO Under Other Abiotic Stresses 127
9.4 NO Signal Transduction in Plants Under Abiotic Stress 128
9.4.1 cGMP-Dependent Signaling 128
9.4.2 Downstream Signaling for NO Action 129
9.5 Interactions of NO Signaling with Other Signaling Molecules in Plant Response to Abiotic Stress 131
References 135
10 Interplay Between Nitric Oxide and Other Signals Involved in Plant Resistance to Pathogens 139
*Jolanta Floryszak-Wieczorek and Magdalena Arasimowicz-Jelonek*

10.1 Introduction 139
10.2 NO Burst 140
10.3 Cooperation of NO with H$_2$O$_2$ in Triggering Programmed Cell Death 142
10.4 Cross Talk of NO with Salicylic Acid, Jasmonic Acid, and Ethylene 145
10.5 The Role of NO in the Micro- and Macroscale of Plant Communication 146

10.5.1 NO Cell Signaling Domain 147
10.5.2 NO in Short-Distance Communication 147
10.5.3 NO from Cross- to Long-Distance Communication 148
10.6 Does NO Participate in Stressful Memory of the Plant? 149
10.7 NO and Plant Recovery from Stress 151
10.8 NO in the Offensive Strategy of the Pathogen 154
10.9 Concluding Remarks 155

References 155

11 Nitric Oxide Signaling by Plant-Associated Bacteria 161
*Michael F. Cohen, Lorenzo Lamattina, and Hideo Yamasaki*

11.1 Introduction 161
11.2 Production of Nitric Oxide by Bacteria 162
11.2.1 Nitrification 162
11.2.2 Denitrification 163
11.2.3 Nitric Oxide Synthase 164
11.3 Regulatory Roles for Nitric Oxide in Bacteria 164
11.3.1 Metabolic Regulation 164
11.3.2 Regulation of Biofilm Formation 165
11.3.3 Stimulation of Oxidative and Nitrosative Defenses 165
11.4 Bacterial Nitric Oxide in Plant–Bacteria Interactions 166
11.4.1 Production of NO in Response to Plant Products 166
11.4.2 Plant Responses to Bacterial NO: The *Azospirillum*–Tomato Interaction 166
11.4.3 Perspectives 169

References 169

12 Nitric Oxide Synthase-Like Protein in Pea (*Pisum sativum* L.) 173
*Mui-Yun Wong, Jengsheng Huang, Eric L. Davis, Serenella Sukno, and Yee-How Tan*

12.1 Introduction 173
12.2 Physiological and Immunoblot Analyses of NOS-Like Protein of Pea 174
12.3 Isolation and Characterization of an NOS-Like Protein of Pea 177
12.4 Molecular Cloning and Analyses of an NOS-Like Gene of Pea 181
12.5 Correlation Study of NOS-Like Gene Expression and NOS Activity in Compatible and Incompatible Pea–Bacteria Interactions 184
References 185

13  Posttranslational Modifications of Proteins by Nitric Oxide: A New Tool of Metabolome Regulation 189
Jasmeet Kaur Abat and Renu Deswal 189
13.1 Introduction 189
13.2 S-Nitrosylation 190
13.2.1 S-Nitrosylation and Ethylene Biosynthesis 191
13.2.2 S-Nitrosylation and Photosynthesis 192
13.2.3 S-Nitrosylation and Glycolysis 194
13.2.4 S-Nitrosylation and Biotic/Abiotic Stresses 195
13.3 Tyrosine Nitration 197
13.4 Binding to Metal Centers 198
13.5 Conclusions and Prospects 198
References 200

Index 203
Preface

The role of nitric oxide (NO) in biological systems has experienced increased prominence in the scientific literature since the 1980s, particularly after coming to light as a signaling molecule in plants in the late 1990s. The number of publications concerning the influence of nitric oxide in plants has dramatically increased since then. Nitric oxide is an easily diffused bioactive and signal-transmitting molecule that directly regulates many plant functions including germination, leaf expansion, root growth, stress physiology, and sequential cell death. This molecule also participates in the adaptation of plants to environmental stresses, working as the key signal carrier in defense response. Recent studies have shown that nitric oxide imparts synergistic effects with phytohormones in physiological regulation and signal transmission.

The purpose of this work is to present recent advances in the role of nitric oxide on plant physiology. This book, composed of 13 chapters contributed by scholars worldwide, addresses mechanisms of NO action in specific plant physiological processes and application of instrumentation for assessing such actions. Chapter 1 embodies recent discoveries in NO chemical properties and the mechanism of NO biosynthesis with special emphasis on the role of nitric oxide in physiological and biochemical changes that occur in plants under normal conditions due to exogenously applied or endogenously produced nitric oxide. Also presented is the issue of cross talk between nitric oxide and other phytohormones. In Chapter 2, electron paramagnetic resonance (EPR) is discussed as a tool to study nitric oxide generation in plants. Recent progress in nitric oxide research with respect to calcium and signaling is discussed in Chapter 3. The current knowledge of nitric oxide in plants exposed to diverse environmental stresses such as salinity, heavy metals, UV-B radiation, ozone, and mechanical wounding comprises Chapter 4. Chapter 5 deals with nitric oxide biosynthesis in relation to polyamines and cytokinins. Moreover, there are a number of similarities in cytokinin- and polyamine-mediated physiological functions such as embryogenesis, flowering, and senescence and in plant responses to abiotic and biotic stresses, which indicate overlapping functions of both these signaling substances; this is also discussed in Chapter 5. The role of nitric oxide in cadmium-induced PCD is discussed in Chapter 6, suggesting a possible
regulatory role in response to heavy metal stress. Chapter 7 focuses on how research on nitrate reductase-deficient plants may contribute to the elucidation of mechanisms involved in nitric oxide production and signaling during plant–pathogen interactions. The function of nitric oxide in plant–pathogen interactions is discussed in detail in Chapter 8. In Chapter 9, various nitric oxide functions as a bioactive signaling molecule in plant abiotic stress responses are discussed. The cross talk between NO and other key signaling components under abiotic stress is also reviewed. The role of NO in the recovery from disease related to the stimulation of wound-healing processes is discussed in Chapter 10. Nitric oxide signaling by plant-associated bacteria is discussed in Chapter 11. Chapter 12 deals with nitric oxide synthase (NOS) activity in pea. The possibility of NO production from various sources in pea cells is also discussed. Chapter 13 describes posttranslational modifications of protein by nitric oxide.

This book is not intended to serve as an encyclopedic review of the subject of NO and its role in plant physiology; however, the various chapters incorporate both theoretical and practical aspects and serve as a baseline information for future research. It is hoped that this book will be useful to students, teachers, and researchers, both in universities and in research institutes, especially in the field of biological and agricultural sciences.

With great pleasure, we extend our sincere thanks to all contributors for their timely response, their excellent and up-to-date contributions, and consistent support and cooperation. We are also thankful to Dr. Zaki A. Siddiqui and Dr. Qazi Fariduddin, Department of Botany, Aligarh Muslim University, Aligarh, India for their encouragement. We are extremely thankful to Wiley-VCH, Germany, for expeditious acceptance of our proposal and completion of the review process. Subsequent cooperation from Wiley-VCH staff is also gratefully acknowledged. We express our sincere thanks to the members of our families for all the support they provided and the neglect and loss they suffered during the preparation of this book.

Finally, we are thankful to the Almighty who provided and guided all the channels to work in cohesion and coordination right from the conception of the idea to the development of the final version of this treatise, “Nitric Oxide in Plant Physiology.”

Shamsul Hayat
Masaki Mori
John Pichtel
Aqil Ahmad
List of Contributors

**Jasmeet Kaur Abat**
University of Delhi  
Department of Botany  
Plant Molecular Physiology and Biochemistry Laboratory  
Delhi 110007  
India

**Aqil Ahmad**  
Higher College of Technology  
Department of Applied Sciences  
Al-Khuwair  
Sultanate of Oman  

and  
Aligarh Muslim University  
Department of Botany  
Aligarh 202002  
India

**Magdalena Arasimowicz-Jelonek**  
Adam Mickiewicz University  
Faculty of Biology  
Department of Plant Ecophysiology  
Umultowska 89  
61-614 Poznan  
Poland

**Juan B. Barroso**
Universidad de Jaén  
Área de Bioquímica y Biología Molecular  
Unidad Asociada al CSIC (EEZ)  
Grupo de Señalización Molecular y Sistemas Antioxidantes en Plantas  
Jaén  
Spain

**Alejandro D. Boveris**
University of Buenos Aires  
School of Pharmacy and Biochemistry  
Physical Chemistry-PRALIB  
Buenos Aires  
Argentina

**Marcia Regina Braga**
Instituto de Botânica (IBt)  
Seção de Fisiologia e Bioquímica de Plantas  
CP 3005  
01061-970 São Paulo, SP  
Brazil

**Michael F. Cohen**
Sonoma State University  
Department of Biology  
Rohnert Park, CA  
USA
List of Contributors

**Francisco J. Corpas**  
Estación Experimental del Zaidín (EEZ), CSIC  
Departamento de Bioquímica, Biología Celular y Molecular de Plantas  
Granada  
Spain

**Alex Costa**  
University of Padova  
Department of Biology  
Via U. Bassi 58/B  
35131 Padova  
Italy

**Eric L. Davis**  
North Carolina State University  
Department of Plant Pathology  
Raleigh, NC 27695-7616  
USA

**Luis A. del Río**  
Estación Experimental del Zaidín (EEZ), CSIC  
Departamento de Bioquímica, Biología Celular y Molecular de Plantas  
Granada  
Spain

**Renu Deswal**  
University of Delhi  
Department of Botany  
Plant Molecular Physiology and Biochemistry Laboratory  
Delhi 110007  
India

**Qazi Fariduddin**  
Aligarh Muslim University  
Department of Botany  
Plant Physiology Section  
Aligarh 202002  
India

**José A. Feijó**  
Instituto Gulbenkian de Ciência  
Centro de Biologia do Desenvolvimento  
Oeiras  
Portugal

**Roberto De Michele**  
University of Padova  
Department of Biology  
Via U. Bassi 58/B  
35131 Padova  
Italy

and

**Universidade de Lisboa**  
Faculdade de Ciências  
Dept Biologia Vegetal  
Campo Grande  
Lisboa  
Portugal

**Jolanta Floryszak-Wieczorek**  
Poznan University of Life Sciences  
Department of Plant Physiology  
Wołyńska 35  
60-637 Poznan  
Poland

**Gang-Ping Hao**  
Taishan Medical University  
Department of Biological Science  
Taian 271000  
China

**Halley Caixeta de Oliveira**  
Universidade Estadual de Campinas – UNICAMP  
Instituto de Biologia  
Departamento de Bioquímica  
CP 6109  
13083-970 Campinas, SP  
Brazil
Syed Aiman Hasan  
Aligarh Muslim University  
Department of Botany  
Plant Physiology Section  
Aligarh 202002  
India  

Shamsul Hayat  
Aligarh Muslim University  
Department of Botany  
Plant Physiology Section  
Aligarh 202002  
India  

Jengsheng Huang  
North Carolina State University  
Department of Plant Pathology  
Raleigh, NC 27695-7616  
USA  

Sebastián Jasid  
University of Buenos Aires  
School of Pharmacy and Biochemistry  
Physical Chemistry-PRALIB  
Buenos Aires  
Argentina  

Lorenzo Lamattina  
Universidad Nacional de Mar del Plata  
Instituto de Investigaciones Biológicas  
Facultad de Ciencias Exactas y Naturales  
CC 1245  
7600 Mar del Plata  
Argentina  

Marina Leterrier  
Estación Experimental del Zaidín (EEZ),  
CSIC  
Departamento de Bioquímica, Biología Celular y Molecular de Plantas  
Granada  
Spain  

Fiorella Lo Schiavo  
University of Padova  
Department of Biology  
Via U. Bassi 58/B  
35131 Padova  
Italy  

Luzia V. Modolo  
Universidade Federal de Minas Gerais  
Instituto de Ciências Biológicas  
Departamento de Botânica  
Belo Horizonte, MG 31270-901  
Brazil  

Masaki Mori  
National Institute of Agrobiological Sciences  
Division of Plant Sciences  
Plant Disease Resistance Unit  
2-1-2, Kannondai, Tsukuba  
Ibaraki 305-8602  
Japan  

José M. Palma  
Estación Experimental del Zaidín (EEZ),  
CSIC  
Departamento de Bioquímica, Biología Celular y Molecular de Plantas  
Granada  
Spain
D. Marshall Porterfield
Purdue University
Bindley Bioscience Center
Physiological Sensing Facility
Discovery Park
West Lafayette, IN
USA

and

Purdue University
Department of Agricultural & Biological Engineering
West Lafayette, IN
USA

and

Purdue University
Department of Horticulture & Landscape Architecture
West Lafayette, IN
USA

Ione Salgado
Universidade Estadual de Campinas – UNICAMP
Instituto de Biologia
Departamento de Bioquímica
CP 6109
13083-970 Campinas, SP
Brazil

Günter F. E. Scherer
Leibniz Universität Hannover
Institut für Zierpflanzenbau und Gehölzforschung
Abt. Molekulare Ertragsphysiologie
Herrenhäuser Str. 2
30419 Hannover
Germany

Ana Margarida Prado
Instituto Gulbenkian de Ciência
Centro de Biologia do Desenvolvimento
Oeiras
Portugal

Serafina Sukno
Universidad de Salamanca
Centro Hispano-Luso de Investigaciones Agrarias (CIALE)
Departamento de Microbiología y Genética
Campus de Villamayor C/Río Duero 12
37185 Villamayor
España

Marcela Simontacchi
University of Buenos Aires
School of Pharmacy and Biochemistry
Physical Chemistry-PRALIB
Buenos Aires
Argentina

Susana Puntarulo
University of Buenos Aires
School of Pharmacy and Biochemistry
Physical Chemistry-PRALIB
Buenos Aires
Argentina

Yee-How Tan
Universiti Putra Malaysia
Faculty of Agriculture
Department of Plant Protection
43400 Serdang
Malaysia
Rinukshi Wimalasekera
Leibniz Universität Hannover
Institut für Zierpflanzenbau und
Gehölzforschung
Abt. Molekulare Ertragsphysiologie
Herrenhäuser Str. 2
30419 Hannover
Germany

Mui-Yun Wong
Universiti Putra Malaysia
Institute of Tropical Agriculture
43400 Serdang
Malaysia

and

Universiti Putra Malaysia
Faculty of Agriculture
Department of Plant Protection
43400 Serdang
Malaysia

Hideo Yamasaki
University of the Ryukyus
Faculty of Science
Nishihara, Okinawa 903-0213
Japan

Jianhua Zhang
Hong Kong Baptist University
Department of Biology
Hong Kong
China

Michela Zottini
University of Padova
Department of Biology
Via U. Bassi 58/B
35131 Padova
Italy

and

Universidad Autònoma de Barcelona
Departamento de Bioquímica y Biología Molecular
08193 Bellaterra, Barcelona
Spain
Nitric Oxide: Chemistry, Biosynthesis, and Physiological Role
Shamsul Hayat, Syed Aiman Hasan, Masaki Mori, Qazi Fariduddin, and Aqil Ahmad

Summary

NO is recognized as a biological messenger in plants. It is a highly reactive gaseous free radical, soluble in water and lipid. It can be synthesized in plants via different enzymatic and nonenzymatic sources such as NOS, NR, XOR, and Ni-NOR. Due to its high lipophilic nature, it can easily diffuse through membrane and can act as an inter- and intracellular messenger and regulate diverse physiological and biochemical processes in plants in a concentration-dependent manner, such as seed dormancy, growth and development, senescence, respiration, photosynthesis, programmed cell death, antioxidant defense system, and so on. Moreover, NO also has an ability to act simultaneously with other molecules and signals in plants. This chapter covers the advances in chemical properties and mechanism of its biosynthesis with special emphasis on the role of NO in the physiological and biochemical changes that occur in plants under normal conditions due to the exogenously applied or endogenously produced NO, along with the cross talk between NO and other phytohormones.

1.1 Introduction

Since the past decade, nitric oxide (NO) is recognized as a novel biological messenger in plants and animals and has received special attention from most of the branches of biological sciences, including medicine, biochemistry, physiology, and genetics. The interest of biologists gained special momentum when this highly reactive radical was identified as a potent endogenous vasodilator of the endothelium [1]. Moreover, a widespread biological significance of nitric oxide was first recognized by Koshland [2] who named this free radical as “Molecule of Year.” The 1998 Nobel Prize in Physiology and Medicine was awarded for the discovery of NO as a biological mediator produced in mammalian cells.
The role of NO is not confined only to the animal kingdom, but plants also have
the ability to accumulate and metabolize atmospheric NO. Klepper [3] for the first
time observed the production of NO in soybean plant, treated with photosynthetic
inhibitor herbicides [4, 5], other chemicals [6, 7], or under anaerobic conditions
[6, 8]. In plants, NO can be generated via enzymatic and nonenzymatic pathways.
The enzymatic pathway is catalyzed by cytosolic nitrate reductase (cNR), NO
synthase (NOS) or NOS-like enzymes, and nitrite:NO reductase (Ni-NOR).
Nonenzymatic pathway is nitrite dismutation to NO and nitrate at acidic pH
values [9–11].

After the discovery of the existence of NO in plant, the question arose, should NO
be considered a phytohormone or not because the classical concept of hormone is
based on three premises [12]: (i) localized site of biosynthesis, (ii) transport to target
cells especially separated from the place of synthesis, and (iii) control of responses
through changes in endogenous levels of the chemical. First, NO had been found to
be formed mainly in actively growing tissues such as embryonic axes and cotyledons,
and the level decreases in mature and senescing organs [13, 14]. Second, the smaller
size of the molecule and its higher diffusion rate through biological membranes
mean that NO fits the premise that hormones are easily transported. Third, it is the
sensitivity of the target cells, rather than the concentration of the plant hormone, that
defines the magnitude of a response [15]; because of this concept, some scientists
decided to substitute the term hormone with a wider term, “plant growth regulator.”
Later on, Beligni and Lamattina [16] categorized NO as a nontraditional regulator of
plant growth.

Further investigations led to the finding that NO is soluble in water and lipid. It can
exist in three interchangeable forms: the radical (NO·), nitrosonium cation (NO+),
and nitroxyl anion (NO−). Due to its high lipophilic nature, NO may diffuse through
membranes [17] and acts as an inter- and intracellular messenger in many physiological functions. It plays a significant role in plant growth and development, seed
germination, flowering, ripening, and senescence of organs [18]. Moreover, like other
phytohormones, NO also acts in a concentration-dependent manner.

Research on NO in plants has gained a considerable attention in recent years and
there is increasing evidence corroborating the role of this molecule in plants.
Therefore, in this chapter, an effort has been made to cover the recent advances
in chemical properties and mechanism of its biosynthesis with special emphasis on
the role of NO in physiological and biochemical changes that occur in plants under
normal conditions due to the exogenously applied or endogenously produced NO,
along with the cross talk between NO and other phytohormones.

1.2
Nitric Oxide Chemistry

Nitric oxide is a gaseous free radical; its chemistry implicates an interplay between the
three redox-related species: nitric oxide radical (NO·), nitrosonium cation (NO+),
and nitroxyl anion (NO−). In biological systems, NO· reacts rapidly with atmospheric
oxygen ($O_2$), superoxide anion ($O_2^{•−}$), and transition metals. The reaction of NO$^•$ with $O_2$ results in the generation of NO$\_x$ compounds (including NO$^•_2$, N$_2$O$_3$, and N$_2$O$_4$), which can either react with cellular amines and thiols or simply hydrolyze to form the end metabolites nitrite (NO$^−_2$) and nitrate (NO$^−_3$) [19]. The reaction of NO$^•$ with O$_2^{•−}$ yields peroxynitrite (ONOO$^−$), a powerful oxidant that mediates cellular injury. At physiological pH, ONOO$^−$ equilibrates rapidly with pernitrous acid (ONOOH) that, depending on its conformation, rapidly decomposes to NO$^−_3$ or to the highly reactive hydroxyl radical HO$^•$. NO$^•$ also forms complexes with transition metals found in heme- or cluster-containing proteins, thus forming iron–nitrosyl complexes. This process alters the structure and function of the target proteins, as exemplified by the activation of soluble guanylate cyclase and the inhibition of aconitases.

In addition, NO$^•$ is extremely susceptible to both oxidation and reduction. One-electron oxidation of NO$^•$ leads to the formation of NO$^+$(nitrosonium cation), while the product of one-electron reduction of NO$^•$ is a nitroxyl anion (NO$^−$) [20–22]. This oxidation can be supported by Fe(III)-containing metalloproteins [20, 21]. NO$^+$ mediates electrophilic attack on reactive sulfur, oxygen, nitrogen, and aromatic carbon centers, with thiols being the most reactive groups. This chemical process is referred to as nitrosation. Nitrosation of sulphydryl (S-nitrosation) centers of many enzymes or proteins has been described and the resulting chemical modification affects the activity in many cases. Such modifications are reversible and protein S-nitrosation–denitrosation could represent an important mechanism for regulating signal transduction. One-electron reduction of NO$^•$ generates NO$^−$. The physiological significance of NO$^−$ has not been clarified. Some workers [20, 23] suggest that it could act as a stabilized form of NO. NO$^−$ is also believed to react with Fe(III) heme and to mediate sulphydryl oxidation of target proteins.

1.3 Biosynthesis of Nitric Oxide

In biological systems, NO can be formed both enzymatically and nonenzymatically. The enzyme responsible for NO generation in animals is nitric oxide synthase. Although NOS-like activity has been detected widely in plants, animal-type NOS is still elusive. Recently, in pea seedlings, using the chemiluminescence assay, Corpas et al. [24] showed arginine-dependent NOS activity, which was constitutive, sensitive to an irreversible inhibitor of animal NOS, and dependent on the plant organ and its developmental stage.

A gene encoding NOS-like protein AtNOS1 was isolated from the Arabidopsis genome; it is involved in the process of growth and hormonal signaling [25]. It was also observed that AtNOS1 may function as NO source in the process of flowering control [26] and in defense response, induced by a lipopolysaccharide [27]. DNA sequencing analyses did not show affinity of AtNOS1 protein to any of animal-origin NOS isoforms. However, the most recent studies have raised critical questions regarding the nature of AtNOS1 [28, 29]. AtNOS1 (Q664P9) and the orthologous genes from rice (Q6YPG5) and maize (AY110367) have been cloned; however, after