Signaling and Communication in Plants

Kapuganti Jagadis Gupta
Abir U. Igamberdiev *Editors*

Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants

Springer

Signaling and Communication in Plants

Series Editor
František Baluška
Department of Plant Cell Biology, IZMB, University of Bonn, Bonn, Germany

More information about this series at
http://www.springer.com/series/8094

Kapuganti Jagadis Gupta • Abir U. Igamberdiev
Editors

Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants

Volume 23

Springer

Editors
Kapuganti Jagadis Gupta
Department of Plant Sciences
University of Oxford
Oxford, United Kingdom

Abir U. Igamberdiev
Memorial University of Newfoundland
St John's, Newfoundland and Labrador
Canada

ISSN 1867-9048 ISSN 1867-9056 (electronic)
ISBN 978-3-319-10078-4 ISBN 978-3-319-10079-1 (eBook)
DOI 10.1007/978-3-319-10079-1
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014958304

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Reactive oxygen species (ROS) and nitric oxide (NO), which is the main representative of reactive nitrogen species (RNS), are important free radical molecules that are formed as by-products of metabolism and participate in signalling events. ROS and RNS alone or together play role in a wide array of plant processes such as plant–microbe interactions, responses to abiotic stress, stomatal regulation, and a range of developmental processes. Due to their short half-life, high diffusion capacity, and ability to react rapidly with different components in the cell, they participate in various processes connected with signalling and communication in plants.

The spatial and temporal regulation of ROS and RNS production and scavenging is an important aspect of their signalling function. ROS and RNS are produced in plant cell in various compartments that include chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum (ER), and plasma membranes. ROS and RNS fulfil numerous essential functions right from germination of seeds to plant senescence. This book describes different signalling and communication processes governed by these molecules. In the first chapters, starting from the general overview of the editors K.J. Gupta and A.U. Igamberdiev, the production of NO and ROS is discussed in relation to operation of various enzyme systems and their compartmentalization. E. Urarte et al. describe the principal role of xanthine oxidoreductase in production and turnover of ROS and NO in plants. F. Minibayeva and R.P. Beckett particularly focus on the role of plant peroxidases in metabolism of reactive nitrogen species and other nitrogenous compounds. Hypoxia in plants is often associated with flooding and represents an important topic relevant to plant productivity. In this context, K.H. Hebelstrup and I.M. Møller review the data on ROS and RNS signalling between mitochondria and the rest of the cell under hypoxic conditions. Plants also contain various scavenging systems for ROS and RNS. The extent of ROS and RNS formation and their level depend on their production and scavenging systems. V.N. Popov provides the overview of ROS production in mitochondria and of their role in induction of the systems that participate in ROS avoidance and scavenging. If ROS are produced in high concentrations they can be toxic for cellular growth and metabolism, and in the next

chapter V. Mittova et al. focus on the operation of various antioxidant systems in wild and cultivated varieties of tomato, showing the importance of ROS scavenging systems in stress tolerance.

Nitrogen is an essential component of proteins. Plants assimilate nitrogen from soil in the form of nitrate or ammonium. In some soils ammonium concentrations are very high. Ammonium toxicity is associated with redox imbalance and increased ROS levels. In this context, A. Podgórska and B. Szal describe the connection between NH_4^+ nutrition, ROS-producing reactions, and antioxidant systems. Plant organisms, when they are subjected to allelopathic compounds, respond by induction of oxidative stress, manifested as overproduction of ROS and alteration in cellular antioxidant systems. This is discussed in the chapter of A. Gniazdowska et al.

Seed germination is a developmental stagein which plant life originates from the quiescent embryo. There are various events associated with germination ranging from the initial uptake of water by dry seed to the emergence of radicle through the seed coat. Seed germination is accompanied by intensive production of ROS (superoxide anion, hydrogen peroxide, etc.) and RNS (NO and its derivatives). In this context, N.V. Bykova et al. provide a detailed overview of ROS and RNS in bioenergetics, metabolism, and signalling processes associated with seed germination. M. Elhiti and C. Stasolla summarize the information related to the role of ROS homeostasis and signalling in the induction and development of in vitro produced embryos. U. Krasuska et al. Describe the pathways of NO biosynthesis in germinating seeds, potential modes of NO action, and its cross-talk with plant growth regulators that determine seed dormancy and germination.

Nitric oxide participates in cell signalling via post-translational modification of various proteins. One of the key processes is S-nitrosylation. NO signalling in the nucleus is important due to activation of various genes involved in plant response to biotic and abiotic stress and in plant development. In this regard, A. Sehrawat and R. Deswal provide the information on S-nitrosylation of nuclear proteins and on its role in regulation of gene expression. The chapter of Corpas et al. describes in detail the processes of nitration and S-nitrosylation in plants. *S*-nitrosoglutathione reductase is considered as a key enzyme of the regulation of intracellular levels of *S*-nitrosoglutathione and indirectly also of protein *S*-nitrosothiols. Petřivalský et al. describe the role of this important enzyme in their chapter. ROS and RNS interact and cross-talk with calcium signalling. This information, which is crucial for understanding ROS, RNS communication network, is covered by S. Sharma.

Overall the book aims to cover various important aspects of reactive oxygen and reactive nitrogen species signalling and communication in plants. Respected scientists from several countries have contributed for this book, and the editors are extremely grateful to all contributors. We express our heartfelt gratitude to the technical editors and book publishing staff of Springer for their continuous support and timely advice during the course of the preparation of this volume.

Wellington Square, Oxford — Kapuganti J. Gupta
St. John's, NL, Canada — Abir U. Igamberdiev

Contents

Compartmentalization of Reactive Oxygen Species and Nitric Oxide Production in Plant Cells: An Overview 1
Kapuganti J. Gupta and Abir U. Igamberdiev

Established and Proposed Roles of Xanthine Oxidoreductase in Oxidative and Reductive Pathways in Plants 15
Estibaliz Urarte, Raquel Esteban, Jose Fernando Moran, and Florian Bittner

The Roles of Plant Peroxidases in the Metabolism of Reactive Nitrogen Species and Other Nitrogenous Compounds 43
Farida Minibayeva and Richard P. Beckett

Mitochondrial Signaling in Plants Under Hypoxia: Use of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) 63
Kim H. Hebelstrup and Ian M. Møller

Feedback Loop of Non-coupled Respiration and Reactive Oxygen Species Production in Plant Mitochondria 79
Vasily N. Popov

Antioxidative Systems and Stress Tolerance: Insight from Wild and Cultivated Tomato Species 89
Valentina Mittova, Micha Volokita, and Micha Guy

The Role of Reactive Oxygen Species Under Ammonium Nutrition 133
Anna Podgórska and Bożena Szal

Allelopathic Compounds as Oxidative Stress Agents: Yes or NO 155
Agnieszka Gniazdowska, Urszula Krasuska, Olga Andrzejczak, and Dorota Soltys

The Role of Reactive Oxygen and Nitrogen Species in Bioenergetics, Metabolism, and Signaling During Seed Germination 177
Natalia V. Bykova, Junjie Hu, Zhenguo Ma, and Abir U. Igamberdiev

ROS Signalling in Plant Embryogenesis 197
Mohamed Elhiti and Claudio Stasolla

"Nitrosative Door" in Seed Dormancy Alleviation and Germination . . . 215
Urszula Krasuska, Katarzyna Ciacka, Paulina Andryka-Dudek, Renata Bogatek, and Agnieszka Gniazdowska

Dissecting Nitric Oxide Signalling in Nucleus: Role of S-Nitrosylation in Regulating Nuclear Proteins 239
Ankita Sehrawat and Renu Deswal

Nitration and S-Nitrosylation: Two Post-translational Modifications (PTMs) Mediated by Reactive Nitrogen Species (RNS) and Their Role in Signalling Processes of Plant Cells 267
Francisco J. Corpas, Juan C Begara-Morales, Beatriz Sánchez-Calvo, Mounira Chaki, and Juan B. Barroso

***S*-Nitrosoglutathione Reductase: A Key Regulator of S-Nitrosylation in Plant Development and Stress Responses** 283
Marek Petřivalský, Lucie Kubienová, Tereza Tichá, and Lenka Luhová

Interaction of Calcium Signalling with Reactive Oxygen and Reactive Nitrogen Species 301
Samir Sharma

Compartmentalization of Reactive Oxygen Species and Nitric Oxide Production in Plant Cells: An Overview

Kapuganti J. Gupta and Abir U. Igamberdiev

1 Introduction

In recent years, the evidence has been increasing that reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a vital role in plants by controlling major physiological processes such as growth, development, resistance to biotic and abiotic environmental stimuli, and progression of programmed cell death. ROS and RNS are the by-products of plant metabolism: the various sites for ROS and RNS production include chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum (ER), and plasma membranes. The extent of ROS and RNS formation and their level depend on their production and scavenging systems. The enzymes that generate ROS and the main RNS species nitric oxide (NO) have various localizations and can be found in different cell compartments. The spatial and temporal location of ROS and RNS production is important in signalling. For instance, the mitochondrial ROS play role in retrograde signalling and communication between mitochondria and nucleus, while the mitochondrial NO may regulate ATP production. The plasma membrane-derived ROS participate in signalling during biotic and abiotic stresses, whereas the plasma membrane-originated NO plays role in nitrate sensing. In this chapter we briefly discuss metabolic and signalling roles of ROS and NO in relation to their compartmentalization.

K.J. Gupta (✉)
Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK
e-mail: jagadis.kapuganti@plants.ox.ac.uk

A.U. Igamberdiev
Department of Biology, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3X9
e-mail: igamberdiev@mun.ca

K.J. Gupta, A.U. Igamberdiev (eds.), *Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants*, Signaling and Communication in Plants 23,
DOI 10.1007/978-3-319-10079-1_1

2 Compartmentalization of ROS Production

There are several forms of ROS including free radicals such as $O_2^{\bullet -}$ (superoxide radical), $OH^{\bullet}$ (hydroxyl radical), and non-radical (molecular) forms: H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen). ROS are unavoidable by-products of aerobic metabolism being produced in various cellular compartments including chloroplasts, mitochondria, peroxisomes, plasma membrane, and apoplast. We will overview below the sites of ROS production with the emphasis on superoxide and hydrogen peroxide which are most abundant and have important metabolic and signalling roles (Fig. 1).

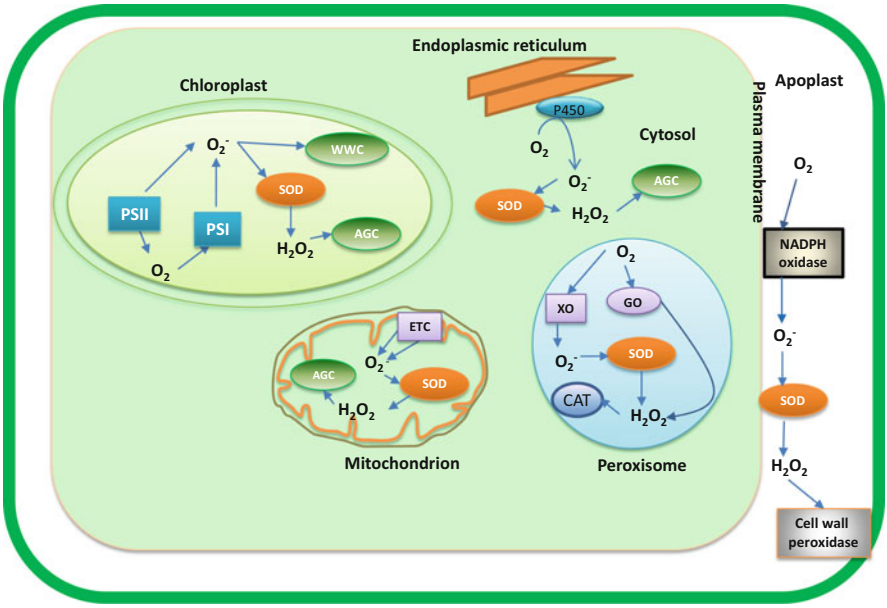

Fig. 1 ROS-generating pathways in various compartments of plant cell. In chloroplasts superoxide production takes place at PSI and PSII; it is converted by SOD to hydrogen peroxide which is scavenged in the ascorbate–glutathione cycle (AGC), also the water–water cycle (WWC) is involved. In plasma membrane NADPH oxidase generates superoxide which is converted by SOD to hydrogen peroxide and the latter is used by cell wall peroxidase. In mitochondria the complexes I and III are sites for ROS production, SOD and AGC are scavengers. In peroxisomes, glycolate oxidase (GO), acyl-CoA oxidase and xanthine oxidase (XO) are major sites of ROS production, catalase (CAT) and SOD are scavengers. In endoplasmic reticulum cytochome P-450 generates superoxide which is scavenged via cytosolic SOD and AGC

2.1 ROS in Chloroplasts

In the light, chloroplasts are the major sites for ROS production due to intensive electron transport during photosynthesis. Both photosystems (PSI and PSII) in chloroplast thylakoids are the major sites for the production of singlet oxygen and superoxide. Electron transport chain (ETC) actively participates in ROS production upon overloading of electron flow in these organelles, which is facilitated by the formation of oxygen in PSII and its abundance. The main reaction associated with ROS production in chloroplasts is Mehler reaction. In this reaction the electron flow is diverted from ferredoxin to O_2, reducing it to superoxide anion. The plastoquinone pool associated with PSII also provides a leakage to O_2 producing superoxide. The singlet oxygen is another ROS which is a by-product of photosynthesis, formed mainly in PSII even under low light. The steady-state level of H_2O_2 in chloroplasts was calculated as ~0.5 μM increasing in stress conditions to 5–15 μM, while the rate of superoxide production is estimated to be ~240 μM s^{-1}, increasing up to 720 μM s^{-1} under stress (Polle 2001). The powerful scavenging mechanisms can keep ROS level under control, but under various stresses, the limitation of carbon fixation in the Calvin cycle leads to a decrease in utilization of NADPH and results in deviation of electrons from ETC to O_2 to form $O_2^{\bullet -}$. The protection from ROS in chloroplasts is achieved by involvement of superoxide dismutases, highly intensive ascorbate–glutathione cycle, chlororespiration, and other mechanisms.

The production of superoxide in PSI becomes an efficient alternative mechanism of photons and electrons sink when it is integrated to be a part of so called water–water cycle, in which SOD and reactions of the ascorbate–glutathione cycle participate (Asada 1999). In the water–water cycle the photoreduction of O_2 to water in PSI takes place by the electrons generated in PSII from water. Its efficiency is based on the intensity of SOD and ascorbate peroxidase activities which are several orders of magnitude higher than the rate of superoxide production. Also the reduction of oxidized forms of ascorbate either by the reduced ferredoxin or by dehydroasorbate and monodehydroascorbate reductases is very fast and efficient. As a result the water–water cycle effectively scavenges photoproduced O_2^- and H_2O_2 and suppresses the production of $OH^{\bullet}$ radicals, thus preventing their interaction with target molecules and hence photoinhibition. Thus, the water–water cycle not only scavenges ROS but also efficiently dissipates excess photon energy and electrons.

2.2 Mitochondria as a Source for ROS

The inner membrane of mitochondria contains protein complexes where electron transfer leads to generation of proton gradient across membrane and this process is coupled to the production of ATP. The reduction level of ubiquinone is directly linked to the leakage of electrons to oxygen which results in formation of superoxide anion. Downstream to the ubiquinone pool two pathways of electron

transport operate. One is the cytochrome pathway via the complexes III and IV and the other is the alternative cyanide-resistant pathway via the alternative oxidase (AOX) which is activated upon the increase of reduction level of ubiquinone and thus prevents overproduction of superoxide (Maxwell et al. 1999). Electron transfer via the cytochrome pathway leads to ATP production but no production of ATP takes place when electrons transfer via AOX pathway due to lack proton pumping sites in the pathway. Under normal respiratory conditions the production of ROS takes place due to the leakage of electrons to oxygen. Under stress conditions this process intensifies and the excess of ROS production takes place. Plants possess various antioxidant systems keeping ROS at low levels but if ROS production exceeds the capacity of antioxidant systems, then ROS become deleterious, causing damage to proteins, lipids and nucleic acids.

In mitochondria the complexes I and III are major sites for ROS production (Møller 2001). The primary electron donor to oxygen for ROS formation is semiubiquinone, however, under stress conditions electrons transfer takes place directly from the complexes I and III to oxygen (Raha and Robinson 2000; Sweetlove and Foyer 2004). The production of ROS in mitochondria occurs when the rate of electron transfer exceeds the capacities of AOX and COX pathways due to generation of excess of electrons (Møller 2001; Rhoads et al. 2006). For instance, the addition of excess of a substrate to mitochondria leads to increased production of ROS (Maxwell et al. 1999). Under stress conditions the ubiquinone pool is over-reduced, which leads to ROS production. Gupta et al. (2014a) has shown that infection of Arabidopsis roots with *Fusarium oxysporum* results in the increased ROS levels due to over-reduction of ubiquinone pool through inhibition of the complex IV by generated NO. Carbon monoxide or cyanide inhibition of complex IV can also increase ROS production in mitochondria (Piantadosi 2008), also the suppression of AOX results in the increased ROS generation (Parsons et al. 1999).

The increased production of ROS leads to lipid peroxidation which can cause cellular damage by reacting with proteins, other lipids, and nucleic acids. The polyunsaturated fatty acids of membrane lipids are prone to ROS production of aldehydes, alkenals, and hydroxyalkenals, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). For instance, 4-hydroxy-2-nonenal (HNE), a toxic product of lipid peroxidation, inhibits oxygen consumption of mitochondria and leads to reduced ATP production (Taylor et al. 2002)

ROS also damage proteins by various mechanisms, e.g. they inhibit both pyruvate dehydrogenase (PDC), 2-oxoglutarate dehydrogenase (OGDC), and glycine decarboxylase (GDC) complexes through modification of lipoic acid moieties. The mechanisms of protein damage by ROS involve (1) Oxidation of various protein moieties such as Cys, Met, Arg, Lys, Pro, and Thr residues (Dean et al. 1997); (2) Cleavage and degradation of protein backbones (Dean et al. 1997); (3) Tyrosine nitration due to joint reaction of ROS and NO (Sehrawat et al. 2013); (4) Direct oxidation of metallic proteins such as aconitase (Verniquet et al. 1991; Gupta et al. 2012). Proteomic approaches have revealed that there are numerous proteins in mitochondria prone to ROS modification (Sweetlove et al. 2002; Kristensen et al. 2004; Taylor et al. 2005). For instance, H_2O_2 or menadione treatments lead to

damage to pyruvate decarboxylase complex, ATP synthase, and various enzymes of the TCA cycle.

Mitochondrial DNA is also damaged by ROS, which has severe consequences in mitochondrial DNA replication and repair. DNA damage also affects retrograde signalling (Rhoads et al. 2006).

2.3 ROS Generation in Peroxisomes

Peroxisomes generate hydrogen peroxide and superoxide anion as a consequence of their metabolic activity involving such processes as the photorespiratory cycle, fatty acid β-oxidation, the glyoxylate cycle, and metabolism of ureides (Corpas et al. 2001; Igamberdiev and Lea 2002). Peroxisomes have extremely high metabolic plasticity in their enzyme composition in development and stress conditions. In leaf peroxisomes, xanthine oxidase (XOD) generates superoxide radicals via oxidation of xanthine or hypoxanthine to uric acid. Superoxide dismutase dismutates superoxide anion to H_2O_2 and catalase degrades H_2O_2. Photorespiratory formation of glycolate by flavin-containing glycolate oxidase in peroxisomes accounts for the majority of H_2O_2 production in leaves (Noctor et al. 2002; Mittler et al. 2004). Catalase is present only in peroxisomes being the most abundant enzyme in these organelles and constituting their core crystal structure, and its function is to scavenge hydrogen peroxide formed mainly in the reactions of flavin-dependent oxidases. The affinity of catalase to H_2O_2 is significantly lower than that of other H_2O_2-scavenging systems, e.g. of ascorbate peroxidase, therefore it can only reduce H_2O_2 level to low millimolar concentrations (Igamberdiev and Lea 2002). Under stress conditions the suppression of catalase in peroxisomes leads to increased production of ROS (del Río et al. 1996).

The peroxisomal membrane is involved in NAD(P)H-dependent O_2^- production in the process of electron transfer involving flavoproteins, NAD(P)H and cytochrome *b* to oxygen (Sandalio et al. 1988; López-Huertas et al. 1999). Monodehydroascorbate reductase (MDHAR) can be an important participant in the balance of ROS in the proximity of peroxisomal membranes by oxidizing NADH and facilitating ascorbate peroxidase reaction (Corpas et al. 2001).

2.4 Plasma Membrane-Mediated ROS Production

Plasma membrane is a major site for ROS production due to presence of NADPH oxidase (Apel and Hirt 2004). This protein is encoded by the respiratory burst oxidase homolog (RBOH) gene family (Torres et al. 2005), it is integral to plasma membrane and composed of six transmembrane domains with two heme groups, C-terminal FAD and NADPH hydrophilic domains and two N-terminal calcium-binding (EF-hand) domains (Marino et al. 2012). The protein is involved in root

hair and pollen development (Potocký et al. 2007; Foreman et al. 2003) and in seed development (Müller et al. 2009), for instance the mutation of Arabidopsis *AtRbohB* leads to altered seed germination (Müller et al. 2009) due to reduced levels of ROS required for cell wall loosening during the germination process. NADPH oxidase is also important in development of programmed cell death (PCD) (Torres et al. 2002). The mechanism of ROS production by NADPH oxidase involves the electron transfer from cytoplasmic NADPH to O_2 to form $O_2^{\bullet -}$, and the latter can be dismutated to H_2O_2.

Mutation in RBOH leads to reduced levels of ROS, failure to induce PCD and to develop the systemic acquired resistance (SAR) in plants (Alvarez et al. 1998). Application of the NADPH oxidase inhibitor diphenyleneiodonium (DPI), leads to reduced production of H_2O_2 (Laloi et al. 2004). NADPH oxidase is involved in signal perception during stress which leads to elevation of calcium and activation of MAP kinases. In tomato RBOH is involved in wounding response (Sagi et al. 2004). The abscisic acid-induced stomatal closure is also associated with RBOH (Kwak et al. 2003). RBOH is also involved in the establishment of symbiotic relations, e.g. of *Medicago truncatula–Sinorhizobium meliloti* symbiotic interaction and nodule functioning (Marino et al. 2011). Downregulation of *MtRbohA* expression leads to a decrease in nodule nitrogen fixation activity. The expression of *MtRbohA* expression is strongly increased under hypoxic conditions (Marino et al. 2011).

Due to operation of RBOH proteins, under many stress conditions the increased ROS accumulation is observed in the apoplast (Hernández et al. 2001). In Arabidopsis guard cells, the apoplast AtRbohD is one of the compartments for ROS production. AtRbohF is another ROS generating enzyme expressed in mesophyll cells of leaves (Kwak et al. 2003). Other candidate enzymes responsible for ROS production in apoplast are polyamine oxidases (Mittler 2002). Apoplastic ROS play role in elongation of leaves which leads to reduced growth under the osmotic stress (Rodríguez et al. 2004).

3 Nitric Oxide Compartmentalization in Plants

Reactive nitrogen species (RNS) are derived from nitric oxide (NO) and include NO itself, peroxynitrite (the product of NO reaction with superoxide) and its derivatives, other reactive nitrogen oxides (NO_2 and N_2O_3). NO-derivatives of small molecules such as nitrosoglutathione are also included in this group. Since the primary process of RNS generation is NO production, we will discuss below localization of production and scavenging of this compound in the plant cell. NO is a free radical that plays role under various biotic and abiotic stresses, growth, and development. There are at least seven pathways responsible for NO production in plants (Gupta et al. 2011), which are classified into the oxidative and reductive pathways. The reductive pathways include participation of the cytosolic nitrate reductase (NR), mitochondrial nitrite NO:reductase, plasma membrane nitrite: NO reductase and xanthine oxidoreductase (XOR),whereas polyamine, hydroxylamine,

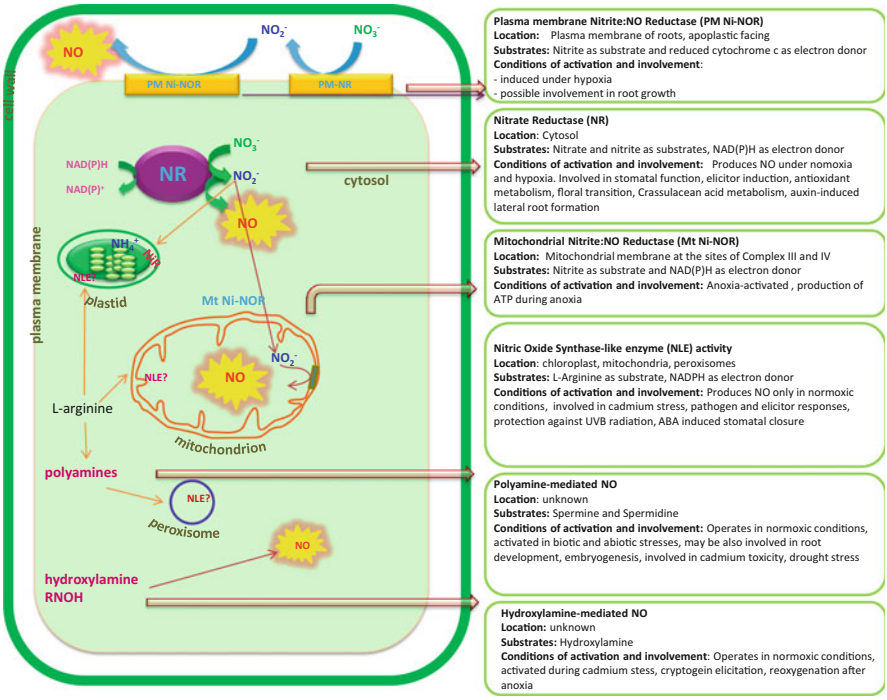

Fig. 2 NO-generating pathways in various compartments of plant cell

and L-arginine-dependent pathways are oxidative in nature. The pathways of NO production and scavenging are located in various compartments and therefore they might fulfil the needs required specifically in each compartment (Fig. 2).

3.1 Cytosolic NO Production

Nitrate reductase (NR) is the major enzyme that is able to produce NO in the cytosol. Using NADH (or NADPH with lower efficiency) as the electron donor it catalyses reduction of nitrate to nitrite, but it can also reduce nitrite to NO (Dean and Harper 1988; Yamasaki and Sakihama 2000). In Arabidopsis NR is encoded by the two genes *Nia1* and *Nia2*. The mutants of NR, *nia1* and *nia2* are impaired in NO production (Gupta et al. 2012). Under various stress conditions accumulation of nitrite takes place which leads to NO formation because nitrite is a limiting factor for NO production (Planchet et al. 2005). Concentration of nitrite should reach high levels to make NR a source of NO generation. The *K*m value for nitrite in NO production by NR is of the order of 100 μM (Yamasaki and Sakihama 2000), which is slightly lower than the *K*m value for nitrite in NO synthesis by mitochondria (175 μM) (Gupta et al. 2005). If the cytosolic pH drops, this leads to inhibition of

nitrite reductase, followed by accumulation of nitrite and NO synthesis. Another factor that influences NO production from NR is its posttranslational modification. Phosphorylation and dephosphorylation regulate NR activity. Phosphorylation of a conserved serine in the NR by NR-kinase results in binding of NR to 14-3-3 proteins, which leads to its inactivation (Lillo et al. 2004). The mutation in phosphorylation site of NR results in the constitutive NO production (Lea et al. 2004).

NR-dependent NO plays an important role under various biotic and abiotic stresses. For instance, NR is the major source of NO production during *Verticillium dahlia* pathogenicity in Arabidopsis (Shi and Li 2008), in *Fusarium oxysporum* infection in Arabidopsis (Gupta et al. 2014a), during *Pseudomonas syringae* infection in tobacco (Modolo et al. 2005; Gupta et al. 2013), in chitosan-induced NO production in guard cells (Srivastava et al. 2009). NR-dependent NO plays a role in several abiotic stresses such as heavy metal stress (Besson-Bard et al. 2009), hypoxia (Gupta et al. 2012), osmotic stress (Kolbert et al. 2010), and cold stress (Zhao et al. 2009). NR-dependent NO is also involved in floral development (Seligman et al. 2008).

3.2 Mitochondrial NO Production

Recent studies have shown that mitochondria are one of the major NO sources and that cytochrome *c* oxidase (COX) and complex III are the plausible sites for NO production in the hypoxic plant cell (Planchet et al. 2005; Stoimenova et al. 2007). The reaction of NO formation at complex IV involves nitrite reduction at the binuclear centre $Fe_{a3}Cu_B$, while the reaction at complex III can be related to leakage of electrons to nitrite from the complex similarly as the leakage to O_2 leads to formation of superoxide (Igamberdiev et al. 2010). The *K*m (nitrite) for this mitochondrial nitrite: NO reductase reaction in roots is 175 μM for root mitochondria, the 50 % inhibition of NO production is observed at 0.05 % O_2 (Gupta et al. 2005). The rates of hypoxic NO production by plant mitochondria are in the range of 1–20 nmol mg^{-1} protein per hour for barley, pea, Arabidopsis (Gupta et al. 2011), however they may be much higher because the most part of NO is immediately scavenged. The scavenging experiments conducted by Gupta et al. (2005) revealed that even under hypoxia mitochondria scavenge 70 % of added NO suggesting that mitochondria are not only producers but also major sinks for NO. NO can also diffuse from mitochondria into cytosol where it is oxygenated to nitrate by the class 1 non-symbiotic hemoglobin (Igamberdiev et al. 2006), which is saturated even at nanomolar oxygen levels. The overall sequence of reactions which includes production of NO from nitrite by mitochondrial complexes III and IV, NO scavenging to nitrate by non-symbiotic hemoglobin, and further reduction of nitrate to nitrite by NR is known as the hemoglobin/nitric oxide (Hb/NO) cycle, playing an important role in bioenergetics of the hypoxic plant cell (Igamberdiev and Hill 2004; Gupta and Igamberdiev 2011).

In animal systems it is shown that COX is not only a source but also an important target for NO (Cleeter et al. 1994). Its inhibition by NO occurs via the competitive binding of NO to the Fe^{2+}-heme group at O_2-binding site of the binuclear centre $Fe_{a3}Cu_B$. This leads to generation of ferrous-heme-nitrosyl complex (Cleeter et al. 1994). The binding has physiological relevance because it keeps oxygen concentration above certain levels and improves oxidative phosphorylation capability (Clerc et al. 2007). COX can scavenge NO back to nitrite during the transition from hypoxia to normoxia (Brunori et al. 2006), and it can also scavenge peroxynitrite formed in the reaction between NO and superoxide (Pearce et al. 2002).

Recently, using the non-symbiotic hemoglobin-overexpressing (nHb+) plants, it was shown that the inhibition of respiration by NO in plants is important for oxygen and ROS homeostasis. Inhibition of COX leads to increase of the internal oxygen concentrations for keeping ROS levels at low level and control of carbohydrate consumption (Gupta et al. 2014b). The reduced levels of NO in nHb+ plants lead to the increased rates of ROS production. A decreased electron flow in the mitochondrial ETC in complex I-deficient plants leads to lower NO production, which affects stomatal conductance and delays growth and morphogenesis (Shah et al. 2013).

In animal systems it was shown that the reduction of nitrite to NO by COX leads to proton translocation (Castello et al. 2006), the same was indirectly established for plants (Stoimenova et al. 2007). It was shown that oxidation of NADH and NADPH under hypoxic conditions leads to low but continuous levels of ATP production, which is very important for hypoxic survival. This reaction was sensitive to myxothiazol and KCN treatment, i.e. the complexes III and IV were involved. It was found that the anoxia-tolerant rice mitochondria produced more NO and ATP under hypoxia than anoxia-intolerant barley mitochondria (Stoimenova et al. 2007). The rates of ATP production were determined as 7–9 nmol min^{-1} mg^{-1} (mitochondrial protein) for barley and 15–17 nmol min^{-1} mg^{-1} (protein) for rice. These rates constitute 3–5 % of the mitochondrial ATP production in ambient oxygen concentrations. The nitrite-dependent ATP generation was insensitive to rotenone suggesting that complex I is not involved in NAD(P)H oxidation in these conditions, and that likely the alternative NADH and NADPH dehydrogenases facing the external site of inner mitochondrial membrane participate in this reaction.

3.3 Peroxisomes as a Source for NO

Plant peroxisomes can be a major site of NO production in the oxidative pathways. It is established that pea leaf peroxisomes generate NO in the nitric oxide synthase-like (NOS like) activity (Barroso et al. 1999). The presence of this reaction was determined based on conversion of L-arginine to citrulline and NO, the level of activity was 170 pmol of L-[^{3}H]citrulline min^{-1} mg^{-1} (peroxisomal protein). This activity was stimulated by calcium, NADPH was required for the reaction and this activity was inhibited by the arginine analogs (Barroso et al. 1999).

Peroxisomes can be involved in NO formation also in the reductive pathways. The peroxisomal enzyme xanthine oxidoreductase (XOR) is able to reduce nitrite to NO (Godber et al. 2000; Corpas et al. 2008). Under hypoxic conditions, the purified XOD was shown to reduce nitrite to NO and this reaction requires NADH or xanthine as electron donors (Corpas et al. 2008). In plants XOR plays a role in NO production in various conditions, e.g. upon phosphate deficiency in cluster roots of white lupin (*Lupinus albus*) (Wang et al. 2010).

3.4 Plasma Membrane NO Production from Nitrite

Plasma membranes of roots contain a protein that possesses nitrite: NO reductase (Ni-NOR) activity. This enzyme has pH optimum at 6.1 (Stöhr et al. 2001). The Ni-NOR has a capacity to reduce the nitrite pool generated in apoplast by the plasma membrane-bound nitrate reductase. Since Ni-NOR is present in plasma membrane, together with plasma membrane NR it has a capacity to sense nitrate in soil, and NO is a signal in this sensing process (Meyer and Stöhr 2002). This enzyme is shown to produce NO in roots in response to infection by mycorrhizal fungi (Moche et al. 2010).

Conclusion
ROS and RNS are the key signalling molecules in plant cells, but when they are produced in excess they cause cellular damage. Under hypoxia, the turnover of nitric oxide complements and partially replaces oxygenic respiration, thus NO serves not only as a signal but also as an important metabolite. The cellular localization of ROS and RNS production and scavenging is based on several organelle-specific systems. Their proper operation is important for keeping the balance of oxidative metabolism in the cell. Any shift of this balance, e.g. under abiotic and biotic stresses, results in switching on mechanisms in which ROS and RNS play a signalling role, and initiating the integrated response with inclusion of hormones, regulatory proteins and responsive genetic elements, which provides adaptation of plants to changing environment.

References

Alvarez ME, Pennell RI, Meijer PJ, Ishikawa A, Dixon RA, Lamb C (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92:773–784

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Barroso JB, Corpas FJ, Carreras A, Sandalio LM, Valderrama R, Palma JM, Lupiáñez JA, del Río LA (1999) Localization of nitric-oxide synthase in plant peroxisomes. J Biol Chem 274:36729–36733

Besson-Bard A, Gravot A, Richaud P, Auroy P, Duc C, Gaymard F, Taconnat L, Renou JP, Pugin A, Wendehenne D (2009) Nitric oxide contributes to cadmium toxicity in Arabidopsis by promoting cadmium accumulation in roots and by up-regulating genes related to iron uptake. Plant Physiol 149:1302–1315

Brunori M, Forte E, Arese M, Mastronicola D, Giuffrè A, Sarti P (2006) Nitric oxide and the respiratory enzyme. Biochim Biophys Acta 1757:1144–1154

Castello PR, David PS, McClure T, Crook Z, Poyton RO (2006) Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. Cell Metab 3:277–287

Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH (1994) Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Lett 345:50–54

Clerc P, Rigoulet M, Leverve X, Fontaine E (2007) Nitric oxide increases oxidative phosphorylation efficiency. J Bioenerg Biomembr 39:158–166

Corpas FJ, Barroso JB, del Río LA (2001) Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. Trends Plant Sci 6:145–150

Corpas FJ, Palma JM, Sandalio LM, Valderrama R, Barroso JB, Del Río LA (2008) Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisumsativum* L.) leaves. J Plant Physiol 165:1319–1330

Dean JV, Harper JE (1988) The conversion of nitrite to nitrogen oxide(s) by the constitutive NAD (P)H-nitrate reductase enzyme from soybean. Plant Physiol 88:389–395

Dean RT, Fu S, Stocker R, Davies MJ (1997) Biochemistry and pathology of radical-mediated protein oxidation. Biochem J 324:1–18

del Río LA, Palma JM, Sandalio LM, Corpas FJ, Pastori GM, Bueno P, López-Huertas E (1996) Peroxisomes as a source of superoxide and hydrogen peroxide in stressed plants. Biochem Soc Trans 24:434–438

Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422:442–446

Godber BL, Doel JJ, Sapkota GP, Blake DR, Stevens CR, Eisenthal R, Harrison R (2000) Reduction of nitrite to nitric oxide catalysed by xanthine oxidoreductase. J Biol Chem 275:7757–7763

Gupta KJ, Igamberdiev AU (2011) The anoxic plant mitochondrion as a nitrite: NO reductase. Mitochondrion 11:537–543

Gupta KJ, Stoimenova M, Kaiser WM (2005) In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. J Exp Bot 56:2601–2609

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT (2011) On the origins of nitric oxide. Trends Plant Sci 16:160–168

Gupta KJ, Shah JK, Brotman Y, Jahnke K, Willmitzer L, Kaiser WM, Bauwe H, Igamberdiev AU (2012) Inhibition of aconitase by nitric oxide leads to induction of the alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J Exp Bot 63:1773–1784

Gupta KJ, Brotman Y, Segu S, Zeier T, Zeier J, Persijn ST, Cristescu SM, Harren FJ, Bauwe H, Fernie AR, Kaiser WM, Mur LA (2013) The form of nitrogen nutrition affects resistance against *Pseudomonas syringae* pv. *phaseolicola* in tobacco. J Exp Bot 64:553–568

Gupta KJ, Mur LAJ, Brotman Y (2014a) *Trichoderma asperelloides* suppresses nitric oxide generation elicited by *Fusarium oxysporum* in *Arabidopsis* roots. Mol Plant Microbe Interact 27:307–314

Gupta KJ, Hebelstrup KH, Kruger NJ, Ratcliffe GR (2014b) Nitric oxide is required for homeostasis of oxygen and reactive oxygen species in barley roots under aerobic conditions. Mol Plant 7:747–750

Hernández JA, Ferrer MA, Jiménez A, Barceló AR, Sevilla F (2001) Antioxidant systems and $O_2^{\bullet -}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127:817–831

Igamberdiev AU, Hill RD (2004) Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. J Exp Bot 55:2473–2482

Igamberdiev AU, Lea PJ (2002) The role of peroxisomes in the integration of metabolism and evolutionary diversity of photosynthetic organisms. Phytochemistry 60:651–674

Igamberdiev AU, Bykova NV, Hill RD (2006) Nitric oxide scavenging by barley hemoglobin is facilitated by a monodehydroascorbate reductase-mediated ascorbate reduction of methemoglobin. Planta 223:1033–1040

Igamberdiev AU, Bykova NV, Shah JK, Hill RD (2010) Anoxic nitric oxide cycling in plants: participating reactions and possible mechanisms. Physiol Plant 138:393–404

Kolbert Z, Ortega L, Erdei L (2010) Involvement of nitrate reductase (NR) in osmotic stress-induced NO generation of *Arabidopsis thaliana* L. roots. J Plant Physiol 167:77–80

Kristensen BK, Askerlund P, Bykova NV, Egsgaard H, Møller IM (2004) Identification of oxidised proteins in the matrix of rice leaf mitochondria by immunoprecipitation and two-dimensional liquid chromatography-tandem mass spectrometry. Phytochemistry 65:1839–1851

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J 22:2623–2633

Laloi C, Apel K, Danon A (2004) Reactive oxygen signalling: the latest news. Curr Opin Plant Biol 7:323–328

Lea US, Ten Hoopen F, Provan F, Kaiser WM, Meyer C, Lillo C (2004) Mutation of the regulatory phosphorylation site of tobacco nitrate reductase results in high nitrite excretion and NO emission from leaf and root tissue. Planta 219:59–65

Lillo C, Meyer C, Lea US, Provan F, Oltedal S (2004) Mechanism and importance of post-translational regulation of nitrate reductase. J Exp Bot 55:1275–1282

López-Huertas E, Corpas FJ, Sandalio LM, Del Río LA (1999) Characterization of membrane polypeptides from pea leaf peroxisomes involved in superoxide radical generation. Biochem J 337:531–536

Marino D, Andrio E, Danchin EG, Oger E, Gucciardo S, Lambert A, Puppo A, Pauly N (2011) A Medicago truncatula NADPH oxidase is involved in symbiotic nodule functioning. New Phytol 189:580–592

Marino D, Dunand C, Puppo A, Pauly N (2012) A burst of plant NADPH oxidases. Trends Plant Sci 17:9–15

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci USA 96:8271–8276

Meyer C, Stöhr C (2002) Soluble and plasma membrane bound enzymes involved in nitrate and nitrite metabolism. In: Foyer CH, Noctor G (eds) Photosynthetic nitrogen assimilation and associated carbon metabolism. Kluwer, Dordrecht, pp 49–62

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Moche M, Stremlau S, Hecht L, Göbel C, Feussner I, Stöhr C (2010) Effect of nitrate supply and mycorrhizal inoculation on characteristics of tobacco root plasma membrane vesicles. Planta 231:425–436

Modolo LV, Augusto O, Almeida IM, Magalhaes JR, Salgado I (2005) Nitrite as the major source of nitric oxide production by *Arabidopsis thaliana* in response to *Pseudomonas syringae*. FEBS Lett 579:3814–3820

Møller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52:561–591

Müller K, Carstens AC, Linkies A, Torres MA, Leubner-Metzger G (2009) The NADPH-oxidase AtrbohB plays a role in Arabidopsis seed after-ripening. New Phytol 184:885–897

Noctor G, Veljovic-Jovanovic S, Driscoll S, Novitskaya L, Foyer CH (2002) Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? Ann Bot 89:841–850

Parsons HL, Yip JYH, Vanlerberghe GC (1999) Increased respiratory restriction during phosphate-limited growth in transgenic tobacco cells lacking alternative oxidase. Plant Physiol 121:1309–1320

Pearce LL, Kanai AJ, Birder LA, Pitt BR, Peterson J (2002) The catabolic fate of nitric oxide: the nitric oxide oxidase and peroxynitrite reductase activities of cytochrome oxidase. J Biol Chem 277:13556–13562

Piantadosi CA (2008) Carbon monoxide, reactive oxygen signaling, and oxidative stress. Free Radic Biol Med 45:562–569

Planchet E, Gupta KJ, Sonoda M, Kaiser WM (2005) Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J 41:732–743

Polle A (2001) Dissecting the superoxide dismutase–ascorbate peroxidase–glutathione pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. Plant Physiol 126:445–462

Potocký M, Jones MA, Bezvoda R, Smirnoff N, Zárský V (2007) Reactive oxygen species produced by NADPH oxidase are involved in pollen tube growth. New Phytol 174:742–751

Raha S, Robinson BH (2000) Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci 25:502–508

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol 141:357–366

Rodríguez AA, Córdoba AR, Ortega L, Taleisnik E (2004) Decreased reactive oxygen species concentration in the elongation zone contributes to the reduction in maize leaf growth under salinity. J Exp Bot 55:1383–1390

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhr R (2004) Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. Plant Cell 16:616–628

Sandalio LM, Fernández VM, Rupérez FL, del Río LA (1988) Superoxide free radicals are produced in glyoxysomes. Plant Physiol 87:1–4

Sehrawat A, Gupta R, Deswal R (2013) Nitric oxide-cold stress signalling cross-talk, evolution of a novel regulatory mechanism. Proteomics 13:1816–1835

Seligman K, Saviani EE, Oliveira HC, Pinto-Maglio CAF, Salgado I (2008) Floral transition and nitric oxide emission during flower development in Arabidopsis thaliana is affected in nitrate reductase-deficient plants. Plant Cell Physiol 49:1112–1121

Shah JK, Cochrane DW, De Paepe R, Igamberdiev AU (2013) Respiratory complex I deficiency results in low nitric oxide levels, induction of hemoglobin and upregulation of fermentation pathways. Plant Physiol Biochem 63:185–190

Shi FM, Li YZ (2008) *Verticillium dahliae* toxins-induced nitric oxide production in Arabidopsis is major dependent on nitrate reductase. BMB Rep 41:79–85

Srivastava N, Gonugunta VK, Puli MR, Raghavendra AS (2009) Nitric oxide production occurs downstream of reactive oxygen species in guard cells during stomatal closure induced by chitosan in abaxial epidermis of *Pisum sativum*. Planta 229:757–765

Stöhr C, Strube F, Marx G, Ullrich WR, Rockel P (2001) A plasma membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from nitrite. Planta 212:835–841

Stoimenova M, Igamberdiev AU, Gupta KJ, Hill RD (2007) Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. Planta 226:465–474

Sweetlove LJ, Foyer CH (2004) Roles for reactive oxygen species and antioxidants in plant mitochondria. In: Day DA, Millar AH, Whelan J (eds) Plant mitochondria: from genome to function, vol 1, Advances in Photosynthesis and Respiration. Kluwer, Dordrecht, pp 307–320

Sweetlove LJ, Heazlewood JL, Herald V, Holtzapffel R, Day DA, Leaver CJ, Millar AH (2002) The impact of oxidative stress on Arabidopsis mitochondria. Plant J 32:891–904

Taylor NL, Day DA, Millar AH (2002) Environmental stress causes oxidative damage to plant mitochondria leading to inhibition of glycine decarboxylase. J Biol Chem 277:42663–42668

Taylor NL, Heazlewood JL, Day DA, Millar AH (2005) Differential impact of environmental stresses on the pea mitochondrial proteome. Mol Cell Proteomics 4:1122–1133

Torres MA, Dangl JL, Jones JDG (2002) Arabidopsis gp91phox homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci USA 99:517–522

Torres MA, Jones JD, Dangl JL (2005) Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in *Arabidopsis thaliana*. Nat Genet 37:1130–1134

Verniquet F, Gaillard J, Neuburger M, Douce R (1991) Rapid inactivation of plant aconitase by hydrogen peroxide. Biochem J 276:643–648

Wang BL, Tang XY, Cheng LY, Zhang AZ, Zhang WH, Zhang FS, Liu JQ, Cao Y, Allan DL, Vance CP, Shen JB (2010) Nitric oxide is involved in phosphorus deficiency-induced cluster-root development and citrate exudation in white lupin. New Phytol 187:1112–1123

Yamasaki H, Sakihama Y (2000) Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. FEBS Lett 468:89–92

Zhao MG, Chen L, Zhang LL, Zhang WH (2009) Nitric reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in Arabidopsis. Plant Physiol 151:755–767

Established and Proposed Roles of Xanthine Oxidoreductase in Oxidative and Reductive Pathways in Plants

Estibaliz Urarte, Raquel Esteban, Jose Fernando Moran, and Florian Bittner

Abbreviations

ABA	Abscisic acid
AO	Aldehyde oxidase
GOGAT cycle	Glutamine oxoglutarate aminotransferase cycle
H_2O_2	Hydrogen peroxide
NO	Nitric oxide
NR	Nitrate reductase
$O_2^{\bullet -}$	Superoxide radical
$ONOO^-$	Peroxynitrite
SOD	Superoxide dismutase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SHAM	Salicylhydroxamic acid
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase

E. Urarte • R. Esteban • J.F. Moran
Institute of Agrobiotechnology, IdAB-CSIC-UPNA-Government of Navarre, 31192 Pamplona, Spain

F. Bittner (✉)
Department of Plant Biology, Braunschweig University of Technology, 38106 Braunschweig, Germany
e-mail: f.bittner@tu-braunschweig.de

K.J. Gupta, A.U. Igamberdiev (eds.), *Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants*, Signaling and Communication in Plants 23,
DOI 10.1007/978-3-319-10079-1_2

1 Introduction

Despite the current description of more than 50 molybdoenzymes in living organisms (Mo-enzymes; Bittner and Mendel 2010), only five of them are found in plants: nitrate reductase (NR), aldehyde oxidase (AO), sulfite oxidase (SO), mitochondrial amidoxime reducing component (mARC), and xanthine oxidoreductase (XOR). This review will focus on XOR, a FAD-, molybdenum-, iron-, and sulfur-containing hydroxylase fundamental in nucleic acid metabolism.

XOR (EC 1.17.1.4., formerly EC 1.1.1.204) is a facultatively oxygen consuming enzyme, which catalyzes the conversion of the purines hypoxanthine and xanthine into uric acid, with the concomitant formation of either NADH or superoxide radical (Bittner and Mendel 2010). The monomer can be subdivided into three distinct domains: a N-terminal domain binding two iron–sulfur clusters of the [2Fe–2S]-type, a domain harboring a flavin adenine dinucleotide (FAD)-binding site, and a C-terminal domain required for molybdenum cofactor (Moco) binding and dimerization. Currently, the crystal structure of plant XOR is not available. However, due to its similarity with bovine, human, and rat XOR, the structure of plant XOR may be assumed based on these models. XOR is a ubiquitous enzyme among the kingdom *Plantae* and for the last three decades it has been deeply studied by different approaches. At early stages, studies with inhibitors like allopurinol provided a useful tool, followed in the last years by *Arabidopsis* knockout plants (Watanabe et al. 2010).

The study on XOR gave origin to the idea of the free radical chemistry in living organisms, mostly based on work by Fridovich and Handler on O_2 consumption by XOR (1958). 10 years later McCord and Fridovich (1968) envisaged that a free radical product derived from oxygen may be produced in biological systems during the reaction of XOR with O_2. The importance of this enzyme lies thus on the fact that it participates in the regulation of reactive oxygen species (ROS) production (Montalbini 1992a). This ROS production in plants by XOR might have outstanding physiological consequences in plant signaling, in the processes of host–pathogen relationships (Montalbini 1992a, b), during abiotic stresses (Zdunek-Zastocka and Lips 2003), or for natural plant senescence (Hesberg et al. 2004). By a common regulatory sulfuration step, the ratio of inactive and active XOR and AO enzymes can be changed rapidly in order to increase the amount of certain important plants hormones such as abscisic acid (ABA) as was shown in drought and salt stressed *Arabidopsis thaliana* (Xiong et al. 2001). Under physiological aspects, this sulfuration step provides an efficient way of regulating the amount of active XOR and AO within the cell and thus, to adopt it to the physiological demands of the plant (Mendel 2011).

This chapter reviews recent insights into the study of the enzyme XOR in plants, with a specific focus on the established and possible physiological substrates, inhibitors and feasible function in plants.

2 History and Evolution of XOR

Due to its high abundance in milk and the simplicity of purification procedures, mammalian XOR is among the most-intensively studied enzymes and was originally referred to as the "Schardinger enzyme" that catalyzes the reduction of methylene blue when formaldehyde served as substrate (Schardinger 1902). Nearly two decades later, extracts of yeast and animal tissues were demonstrated to harbor certain substances that are likewise involved in the reduction of methylene blue in milk (Hopkins 1921), and only 1 year later these substances have been identified as hypoxanthine and xanthine, which lead to the renaming of the enyzme into xanthine oxidase (XO) (Morgan et al. 1922; Booth 1935 and references therein). Later, the term XO became representative for a specific condition of the enzyme (along with xanthine dehydrogenase, XDH), and the term xanthine oxidoreductase (XOR) was introduced as a more general and ubiquitous name for this enzyme that is found in all kingdoms of life. In particular mammalian XOR attracted, and still attracts, many researchers due to its implications in ischemia/reperfusion injury and reactive oxygen species (ROS) generation, but also because of its possible involvement in nitric oxide (NO) and peroxynitrite formation, and because of XOR-related diseases such as xanthinuria and hyperuricemia/gout (reviewed by Harrison 2002; Agarwal et al. 2011). Moreover, mammalian XOR was proven to have another distinct function that is associated with the formation of milk-fat droplets but totally unrelated to enzymatic activity of XOR (Vorbach et al. 2002). However, XOR is generally recognized as a key enzyme in purine degradation, where it catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. In primates and many other animals, the latter reaction represents the last step in this pathway, thus demanding excretion of poorly soluble uric acid with the urine to prevent severe consequences due to crystal formation in blood, serum, tissues, and muscles. In plants however, uric acid is likewise the product of XOR catalysis but far away from being the final product of purine degradation (Fig. 1).

Instead, uric acid is further decomposed to allantoin and allantoate, two important nitrogen storage and transport compounds at least in tropical legumes (Smith and Atkins 2002). Subsequently, allantoate is converted to glyoxylate via three enzymatic steps, which include the formation of ureidoglycine and ureidoglycolate and the release of carbon dioxide and ammonia (Werner and Witte 2011; Werner et al. 2013, and references therein). It is assumed that the latter compounds have the potential of being reused in photosynthesis or reassimilated by the photorespiratory glycolate and GOGAT cycles. Remarkably, the XOR substrate xanthine is the first common intermediate in the degradation of all purine nucleotides, thus representing an important bottleneck in this pathway and making XOR a key enzyme in purine catabolism.

In prokaryotes, XOR has been found exclusively in the XDH form and enables bacteria such as *Comamonas acidovorans* to grow on minimal medium supplemented with hypoxanthine or xanthine as the sole source of carbon and nitrogen (Xiang and Edmondson 1996; Ivanov et al. 2004). In contrast, other

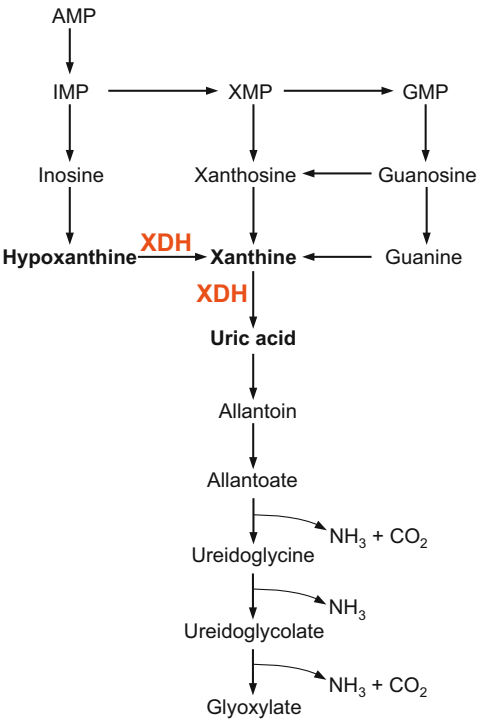

Fig. 1 General scheme of purine degradation in higher plants. The purine degradation pathway is depicted starting with adenosine monophosphate (AMP) at the crosspoint between purine de novo synthesis and catabolism. Only the relevant intermediates are shown according to Zrenner et al. (2006), Werner and Witte (2011) and Werner et al. (2013), presumed back-reactions are not considered. *IMP* inosine monophosphate, *GMP* guanosine monophosphate, and *XMP* xanthosine monophosphate

bacteria like *Escherichia coli* turned out to be unable to live on purines as sole carbon or nitrogen source (Xi et al. 2000), indicating that XOR in these bacteria is involved in purine salvage rather than being part of a complete purine catabolic pathway. The *C. acidovorans* enzyme consists of two 58 kDa α subunits, which each bind two nonidentical [2Fe–2S] iron–sulfur clusters, and two 87 kDa β subunits each binding one FAD and one Moco in its dinucleotide form. The fully constituted and active enzyme thus is an $(\alpha\beta)_2$ heterodimer with a molecular mass of about 290 kDa indistinguishable from the mass of the eukaryotic XOR enzymes. While several other bacteria such as *Rhodobacter capsulatus* (Leimkühler et al. 1998) basically harbor the same type of XOR with identical subunit composition, XOR enzymes from other prokaryotic species such as *Pseudomonas putida*, *Veillonella atypica*, and *Eubacterium barkeri* have been demonstrated to consist of only one or even three subunits, which associate to functionally active enzymes with molecular masses of up to 550 kDa (Woolfolk 1985; Hettrich and Lingens 1991; Gremer and Meyer 1996; Schräder et al. 1999). Differences between these enzymes do also concern variations of the bound redox groups, the composition of redox groups and their oligomerization states, but also the ligands bound to the molybdenum centers as well as the preferred co-substrate, which in some cases is NAD^+ and in others is $NADP^+$. Interestingly, in some bacteria the oxidation of

hypoxanthine to xanthine and of xanthine to uric acid involves two enzymes, with purine hydroxylase catalyzing the first reaction and XOR catalyzing the latter (Self 2002). Yet, among the diverse bacterial XOR forms, the one found in *R. capsulatus* appeared to be more similar to its eukaryotic counterparts than to other prokaryotic XOR enzymes with respect to molecular mass, domain composition, bound redox groups, catalytical properties, and 3D structure (Leimkühler et al. 1998; Truglio et al. 2002), suggesting that eukaryotic XOR has evolved from an ancient bacterial XOR form as found in *R. capsulatus*.

Besides prokaryotes and eukaryotes, XOR homologues can be found also in archaea, with their physiological roles being largely unraveled in most cases. It is noteworthy that XOR is not present in all species but absent in those, which are specialized in a way that the function of XOR is not required (some bacteria and most archaea) or which have adopted to parasitic living styles that either enable the respective organism to acquire all necessary purine intermediates from their host or that have established alternative pathways. In eukaryotes, the loss of XOR genes seems to be accompanied always by the complete loss of molybdenum metabolism as is found in some yeasts species like *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* but not in other yeasts such as *Pichia* (*Komagataella*) *pastoris* (Zhang et al. 2011). Remarkably, the loss of XOR and molybdenum metabolism in eukaryotes appears to be limited to unicellar organisms, whereas multicellular eukaryotes generally appear to have an essential demand for molybdenum metabolism including XOR, which might be ascribed to the specialization of cells and tissues and the need for well-orchestrated pathways.

3 Distribution of XOR Among the Kingdom *Plantae* and Subcellular Localization

XOR occurs ubiquitously within the kingdom *Plantae* and it has been isolated from all organs of plants: root nodules (Triplett et al. 1982; Boland et al. 1983; Nguyen et al. 1986), roots (Barabás et al. 2000), leaves (Nguyen and Feierabend 1978; Montalbini 2000; Vitória and Mazzafera 1999), seedlings and whole plants (Suzuki and Takahashi 1975; Tajima and Yamamoto 1975; Kumar and Taneja 1977; Sauer et al. 2002), and fruits (Vitória and Mazzafera 1999; Taylor and Cowan 2001). Indeed, XOR activity has been reported in a great variety of plant species, including soybean, pea, wheat, maize, tea, lentil, tomato, coffee, alfalfa, avocado, tobacco, and *Arabidopsis* (i.e., Montalbini 1998; Sauer et al. 2002; Nakagawa et al. 2007; Corpas et al. 2009; Shakirov et al. 2012). Despite this widespread distribution and the reasonable number of studies of XOR in plants, the exact subcellular localization has been discussed rather controversially. Initially, XOR was found associated to microbodies (Nguyen 1986), glyoxysomes, and peroxisomes (Sandalio

et al. 1988; Del Río et al. 1989), but later immunolabeling techniques revealed that XOR was present in the cytosol of infected and uninfected cells of legume root nodules, and that this feature was related to the process of ureide biosynthesis (Datta et al. 1991). Parallel and later studies supported the hypothesis of XOR being involved in stress response, and other locations within the plant cell were explored. In this respect, XOR was once more localized in peroxisomes, which are organelles with a potent oxidative metabolism that are additionally well known as ROS and reactive nitrogen species (RNS) generators (Corpas et al. 2008, 2009). In pea leaf peroxisomes, XOR was found to act with both oxidizing substrates, NAD^+ and O_2, where the activity with the latter was surprisingly twofold more pronounced, as indicated by enzymatic assays (Corpas et al. 2008). However, this recent article localizes XOR also in the cytosol, suggesting that XOR has several localizations within the plant cell.

4 Structure of XOR and Its Redox Centers

Unfortunately, no crystal structure of a plant XOR is available until now and a detailed description with regard to structure–function relationships has thus been precluded. Nevertheless, based on the high sequence similarity, the identical domain composition, and nearly identical substrate preferences, the well-known structures of bovine (Enroth et al. 2000), human (Yamaguchi et al. 2007) and rat XOR (Nishino et al. 2005) might well serve as model structures for plant and other eukaryotic XOR enzymes.

All eukaryotic XOR enzymes are homodimeric proteins of 290–300 kDa with each monomer of approximately 145 kDa being constituted of three distinct domains (Fig. 2a). The N-terminal domain of 20 kDa is responsible for binding of two nonidentical iron–sulfur clusters of the [2Fe–2S] type and is followed by a larger middle domain of 40 kDa that binds FAD. The C-terminal domain of 85 kDa exhibits two functions, one of which is the binding of one Moco while the other is to mediate the dimerization of two identical, monomeric XOR polypeptides (Fig. 2b). Iron-sulfur clusters, FAD and Moco are all prosthetic groups that are permanently bound by the enzyme and participate in catalysis and electron transfer. As is known for other molybdenum-dependent enzymes, Moco has a very particular role in forming an essential part of the active site of XOR where substrates are bound and converted and where the respective product is released. Notably, in the crystal structures of mammalian XOR proteins both molybdenum centers are about 50 Å apart, indicating that the two XOR monomers act independently from each other.

Once a reducing substrate such as xanthine or hypoxanthine is bound in the active site of XOR, the oxidative hydroxylation of these substrates takes place at the molybdenum center, whereby the molybdenum becomes protonated and reduced

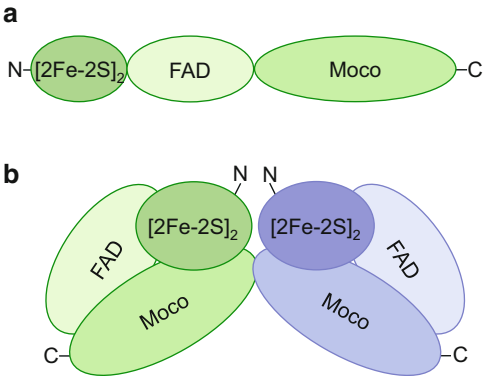

Fig. 2 Schematic presentation of the structure of xanthine oxidoreductase. (**a**) Linear presentation of the domain arrangement in one XOR polypeptide. Each domain is separated from the others by flexible hinge regions. (**b**) Simplified scheme of the three-dimensional structure of eukaryotic XOR according to Enroth et al. (2000). The two monomers of the XOR homodimer are shown in *green* and *blue*, respectively, with the dimerization interface being located within the large Moco domain. In contrast to what the linear structure suggests, electron transfer in the fully folded enzyme is directed from Moco via the two [2Fe–2S] clusters to the FAD, which is enabled by folding of the N-terminal Fe–S cluster domain between the Moco- and FAD-binding domains

from MoVI to MoIV in the reductive half-reaction of the catalytic sequence (Fig. 3). Electrons thus liberated from the substrate are passed on via the two iron–sulfur clusters to the FAD site, where they are finally removed either by NAD^+ or O_2 in the oxidative half-reaction of the catalytic sequence. In this reaction, a particular glutamate residue strictly conserved among all XOR and AO proteins (e.g., Glu1261 in bovine XOR, Glu1297 in *Arabidopsis* AtXDH1) is assumed to function as an active site base required for the activation of substrates (Hille et al. 2011). While the ultimate source of the oxygen atom incorporated by XOR into the hydroxyl group of the respective product has been identified as water (Murray et al. 1966), it has been demonstrated that a hydroxyl ligand of the molybdenum center (Mo–OH) represents the catalytically labile oxygen within the protein (Hille and Sprecher 1987), which is supported by the crystal structure of bovine XOR in which the Mo–OH group points toward the substrate-binding site (Enroth et al. 2000). Accordingly, substrates are coordinated to the catalytically introduced hydroxyl group of the molybdenum atom in a Mo–O–substrate fashion, thereby converting the respective substrate into hydroxylated product (e.g., hypoxanthine into xanthine or xanthine into uric acid, respectively). The product is finally released from the enzyme upon displacement by solvent-derived hydroxide, with electron transfer from the molybdenum center to the iron-sulfur clusters and FAD.