Signaling and Communication in Plants

Kapuganti Jagadis Gupta
Abir U. Igamberdiev *Editors*

Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants

Springer

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Volume 23

Springer

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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
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Contents

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.

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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).

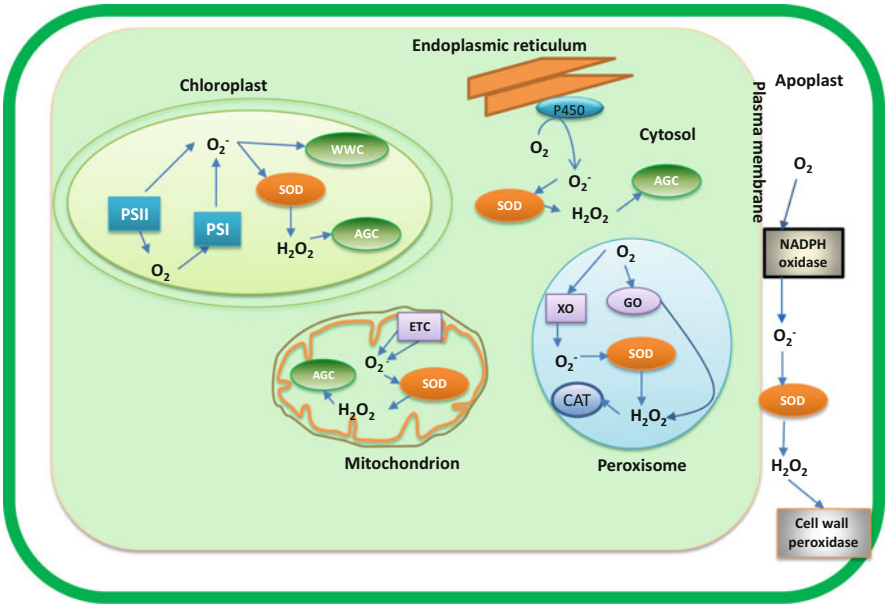

Fig. 1 ROS-generating pathways in various compartments of plant cell. In chloroplasts superoxide production takes place at PSI and PSII; it is converted by SOD to hydrogen peroxide which is scavenged in the ascorbate–glutathione cycle (AGC), also the water–water cycle (WWC) is involved. In plasma membrane 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,

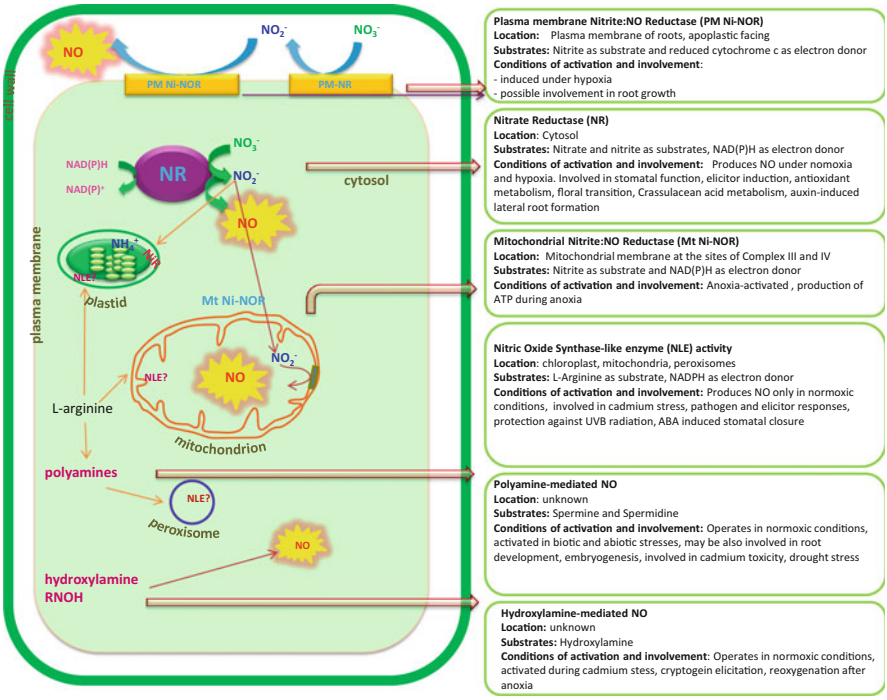

Fig. 2 NO-generating pathways in various compartments of plant cell

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.

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