Nitric Oxide Synthesis in Plants: A Recent Update

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Abstract: In recent years nitric oxide (NO) has been established as a versatile molecule due to its multifaceted roles in growth and development and as a key signaling molecule in different intracellular processes in plants. Moreover, NO also acts as a direct or indirect antioxidant and regulates the level of reactive oxygen species in stressed cells. A plentiful of literature is available with the information on the role of NO in growth, physiological, biochemical and molecular attributes of plants and responses to biotic and abiotic stresses. The enzyme nitric oxide synthase (NOS) that oxidizes arginine to NO and citrulline, has been characterized as the source of NO in animals. However, in plants, as several sources of NO are identified, synthesis of NO is more complex and is a matter of considerable debate. Based on the previous evidences, the present review is focused on the enzymatic and non-enzymatic sources of NO in plants.

Keywords: Nitric oxide, Nitric oxide synthase, Nitrate reductase, Xanthine oxidoreductase.

1. INTRODUCTION

Nitric oxide (NO), a small diffusible, bioactive molecule, was discovered in 1772 by Joseph Priestley as ‘Nitrous Air’. Earlier, it was commonly believed to be only a poisonous gas that causes acid rain. This assumption has prompted the Governments of Canada and the United States to "freeze emissions of nitrogen oxides" in the late 1980s. The prevailing biological significance of nitric oxide gained considerable attention across the world and NO was nominated as ‘Molecule of the Year’ by Science magazine [1]. The importance of NO was further testified when Louis J. Ignerro, Robert F. Furchgott and Ferid Murad identified nitric oxide as a signaling molecule in the cardiovascular system for which they shared the 1998 Nobel Prize for Medicine and Physiology.

Moncanda’s group in 1987, was the first to consider NO as a signaling molecule involved in relaxation of endothelial cells [2-4]. In plants, NO is now well-established as a key signaling molecule. It functions in the control of fundamental processes such as disease resistance [5], mediating responses to abiotic stresses [6], stomatal closure [7], flowering [8], enzyme activities [9], mitogen-activated protein (MAP) kinase signaling pathways [10, 11], expression of cell cycle genes [12], pollen tube re-orientation [13], seed dormancy [14] and germination [15]. The properties and multifaceted roles of NO in plants have developed interest of researchers across the globe in NO synthesis and mechanism of action. In plants, the sources of NO production have been the subject of much debate. A significant number of reports suggest the synthesis of NO by mammalian-like nitric oxide synthase (NOS) activity, nitrate reductase, or non-enzymatic sources. In the light of emerging concepts, in the present review, we highlighted our current understanding of NO biosynthesis in plants.

2. BIOSYNTHESIS OF NITRIC OXIDE IN PLANTS

The generation of NO by plant tissues has long been noted in soybean plants treated with photosynthetic inhibitor herbicides [16] or chemicals as well as under dark anaerobic conditions [17]. Although, several important evidences of NO biosynthesis in plants have been presented during the last few years but it is still not clear that under which condition the NO is produced and how it is regulated. However, it has been proposed that NO can be synthesized both enzymatically and non-enzymatically in plants (Figure **1**).

2.1. Enzymatic synthesis of NO

2.1.1. NO Synthesis by Nitric Oxide Synthase (NOS)

It is well established that nitric oxide synthase (NOS) is the key enzyme responsible for NO synthesis is animal system. This enzyme catalyzes the oxygen- and NADPH-dependent oxidation of L-arginine to NO and citrulline in a complex reaction requiring FAD, FMN, tetrahydrobiopterin (BH4), calcium and calmodulin [18-20]. As in animals, ariginine-dependant activity of NOS was reported in plants [18, 21-23] that can be inhibited by NOS inhibitors. These results were confirmed by the inhibitory action of these inhibitors on NO synthesis and NO-mediated responses. These findings were corroborated by immunological experiments of Ribeiro *et al*. [22], they reported that

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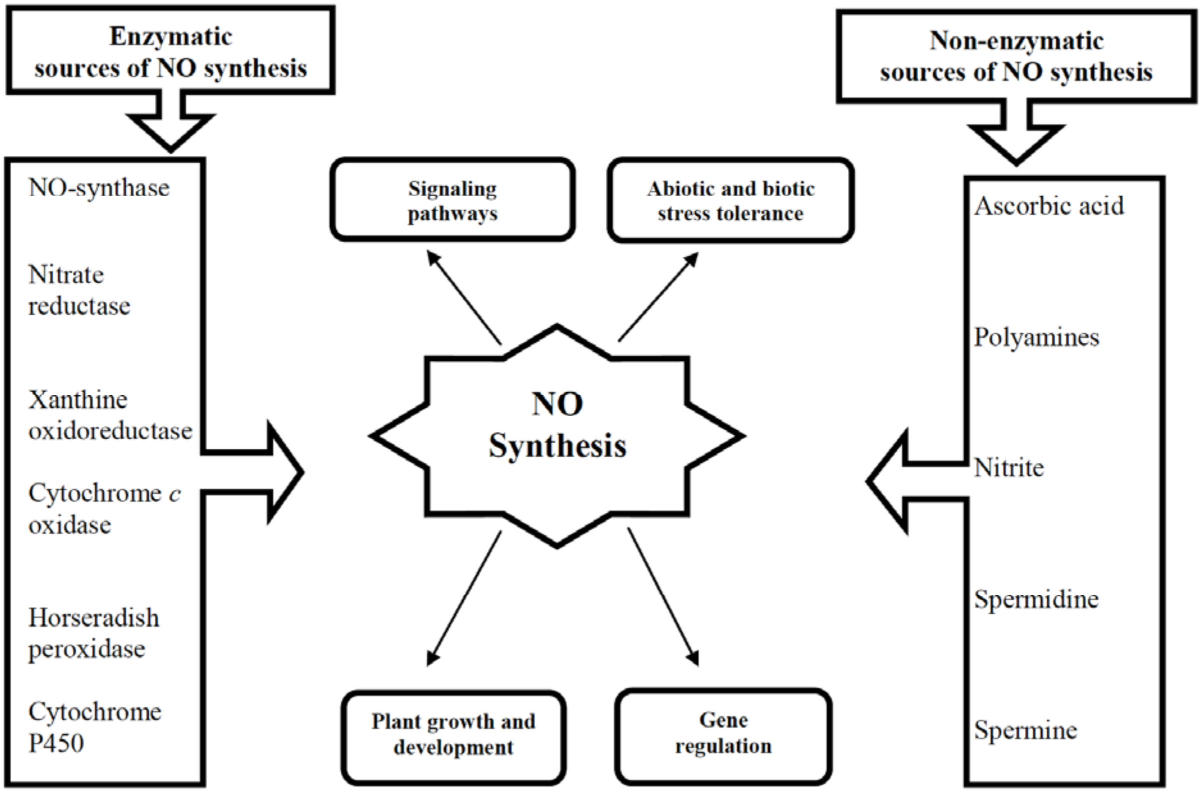

Figure 1: Simplified illustration of the sources of nitric oxide (NO) synthesis in plants.

anti-mammalian NOS antibodies cross-react with plant proteins. On the other hand, Butt *et al*. [19], through proteomic analysis of plant proteins probed by mammalian NOS antibodies, suggested these proteins as heat shock proteins and glycolytic enzymes instead of NOS related ptoteins. Moreover, no protein or gene was identified that had any sequence similarity to the complete animal NOS. There are several studies which claim the presence of NOS activity in plants. The occurrence of NOS activity was reported in pea peroxisomes [24, 25], in roots, stems and leaves of pea seedlings [26], salinity-induced NOS activity in olives [27].

Guo *et al*. [28], identified an *Arabidopsis* protein At-NOS1 that produced NO in response to hormonal signals. At-NOS1 exhibited 16% similarity to a protein from the snail *Helix pomatia*. Moreover, neither the snail protein nor NOS1 were similar to typical animal NOS enzymes, but they increased Arg-dependent NO synthesis. Over-expression of NOS1 in *Arabidopsis* resulted in higher levels of NOS activity in leaf extracts, whereas *Atnos1* mutant, as compared with wild type, displayed lower NOS activity in leaf extracts and reduced NO accumulation in roots. Different groups have independently confirmed the presence of decreased NOS activity and NO levels in the *Arabidopsis nos1* mutant [8, 29, 30]. Guo *et al*. [28] also showed that AtNOS1 has NOS activity dependent on NADPH, Ca^{2+}, and CaM, but not on FAD, FMN or BH4. However, these studies are challenged recently when the researchers [31, 32] were unable to reproduce earlier results. Therefore, it may be postulated that the presence of NOS-like activity in plants is still mysterious.

2.1.2. NO Synthesis by Nitrate Reductase (NR)

Nitrate reductase (NR) is a main enzyme of nitrogen assimilation in plants, with the capacity of NAD(P)H-dependent reduction of nitrite (NO_2^-) to NO [33]. NR is a molybdenum cofactor-containing (Moco) enzyme [34, 35] with rigorously confirmed NO-producing activity both *in vivo* and *in vitro* [36, 33]. NR-dependent NO production has been recorded in cucumber [37], sunflower, spinach and maize [38], *Arabidopsis* [39], wheat, orchid and aloe [40], tobacco [41] as well as in *Chlamydomonas reinhardtii* [42]. Another possible NO_2^-/NO-reductase (NI-NOR) of vascular plants has been identified in the root plasma membrane of the tobacco [43]. NI-NOR reduces NO_2^- to NO using reduced cytochrome c as an electron donor.

It has been sometimes reported that several plant and algal species emit NO when nitrate (NO_3^-) or NO_2^- is supplied in darkness [16, 44, 45]. In Glycine max, the constitutive NR was identified to produce NO [46]. The *in vitro* evidence suggested that maize inducible NR is also capable of producing NO through one electron reduction of NO_2^- [35]. A similar NO_2^--dependent NO production catalyzed by NR has been reported in bacteria [47] and fungi [48]. The NO producing activity of NR is regulated from its high affinity substrate NO_3^- to NO_2^-. Therefore, high NO_2^- levels are required to competitively inhibit NO_3^- reduction through either increased influx of NO_3^- into the vacuole or efflux from the cell. On the other hand, Rockel *et al*. [38], reported low NO production in maize under normal conditions when NO_3^- levels are high and NO_2^- is low. However, under anaerobic conditions when NO_2^- levels were higher, they reported higher values for NO [38]. Two genes for NR, *NIA1* and *NIA2,* were reported from *Arabidopsis* with 83% similarity in their proteins at amino acid level. Deskin *et al*. [39], using double *nia1nia2* mutants showed the involvement of NR in ABA induced generation of NO in guard cells. However, here arises a question that whether *NIA1* or *NIA2* is most significant for NO generation. Hao *et al*. [49], used mutants of *nia1* and/or *nia2*, and showed the involvement of both NR enzymes. NR dependent NO also played an important role in cold and freeze tolerance [50], plant defenses against pathogen attack [51] and initiation of flowering [52].

But still, no conclusive data is available to prove whether NR is directly involved in NO-production in the living cells.

2.1.3. NO Synthesis by Xanthine Oxidoreductase (XOR)

Xanthine oxidoreductase (XOR) is a peroxisome-located enzymes which causes NADH-dependent reduction of NO_2^- to NO under anaerobic conditions [53]. XOR has been reported in pea leaf peroxisomes and exists in two inter-convertible forms: the superoxide-producing xanthine oxidase and xanthine dehydrogenase [54].

Among several plant enzymes, XOR acts as a key enzyme catalyzing the first and rate-limiting reaction of purine breakdown (i.e. oxidation of xanthine to uric acid). This reaction occurs via two possible xanthine-oxidizing activities. The more prominent activity is catalyzed by NAD^+-dependent xanthine dehydrogenase (XDH), while the O_2-reducing xanthine oxidase reaction is much less common and all the plant XOR forms purified so far are of the XDH form [55]. Despite its critical role in plant metabolic pathways, the genomic organization of plant XDH was only recently identified using molecular approaches.

2.1.4. NO Synthesis in Mitochondria

Mitochondria, the powerhouse of the cell, are well known to produce ATP, the energy currency of the cell. Additionally, mitochondria have been shown to produce NO in plant cells [56-58]. The source of NO in plant mitochondria is a matter of extensive debate. The production of NO by a plant NOS has been speculated in the mitochondrion [59]. On the other hand, Gupta *et al*. [58], using the direct and indirect chemiluminescence detection of NO, concluded that plant mitochondria do not contain NOS-like activity, as they could not find any aerobic arginine-dependent NO generation by mitochondria. In contrast, several evidences now exist for NOS in eukaryotic algae such as *Ostreococcus tauri* [59, 60], *Symbiodinium bermudense* [61, 62] and in *Chattonella marina* [63].

Under anaerobic conditions, plant mitochondria have been shown to emit NO from reduction of NO_2^- in presence of NADH [57, 58]. These results strengthen the findings of Tischner *et al*. [56], who reported that under anoxic conditions, NR mutants of *Chlorella*, provided with NO_2^-, release substantial amounts of NO. The terminal cytochrome *c* oxidase of mitochondrial respiratory chain is the site for NO_2^- to NO reduction [64, 58]. However, this reaction was absent in leaf and was observed in root mitochondria [58]. Thus, this might explain that either NO_2^- reduction site in all plant mitochondria are not the same or there exists a difference between the mitochondria of leaf and root. Thus, NOS-like activity in mitochondria is still obscure, whereas, there is confirmed synthesis of NO from NO_2^-.

2.1.5. NO Synthesis in Peroxisomes and by other Sources

Peroxisomes are single-membrane bound organelles in eukaryotic cells, with variable enzymatic contents depending on the organism, cell or tissue type, and environmental conditions [65, 66]. The presence of L-Arg-dependent NOS activity was detected in isolated pea (*Pisum sativum*) leaf peroxisomes [67]. Later on, Corpas *et al*. [68], using electron paramagnetic resonance techniques, demonstrated the presence of NO in these types of peroxisomes. Further, Corpas *et al*. [69], using nitric oxide synthase (NOS) inhibitors detected the presence of putative calcium-dependent NOS activity and showed *in vivo* accumulation of NO in Arabidopsis peroxisomes.

Furthermore, besides above mentioned sources of NO, there are several other enzymatic sources of NO synthesis in plants. Boucher *et al*. [70], reported the generation of NO and citrulline by horseradish peroxidase from N-hydroxyarginine (NOHA) and H_2O_2. Horseradish peroxidase is also able to generate NO and citrulline from hydroxyurea and H_2O_2 [71]. Boucher *et al*. [70], also reported that hemoglobin and catalase cause the oxidation of NOHA by cumyl hydroperoxide to produce NO. The generation of NO by NO_2^- reduction also takes place under hypoxic or anoxic conditions by the mechanism of NO_2^-/NO reducing ability of deoxygenated heme-containing proteins in the peroxisome matrix [72]. Cytochrome P450 are another heme proteins, which have been shown to generate NO by catalyzing the oxidation of NOHA by NADPH and O_2 [73, 74].

2.2. Non-Enzymatic Synthesis of NO

Plants can also synthesize NO by non-enzymatic mechanisms. Yamasaki [48], proposed a non-enzymatic mechanism for the synthesis of NO from NO_2^- under acidic conditions:

$$2NO_2^- + 2H^+ \leftrightarrow 2HNO_2 \leftrightarrow NO + NO_2 + H_2O \leftrightarrow 2NO + \frac{1}{2}2O_2 + H_2O$$

In this reaction nitrite is protonated to form nitrous acid (HNO_2) in a completely reversible reaction at low pH. The non-enzymatic generation of NO cannot occur in cytosolic environments of plant cells because the pH of the cytoplasm strongly prevents the formation of HNO_2 [48]. Furthermore, it has been noted that reducing agents like ascorbic acid and phenolics can accelerate the rate of formation of NO.

Nagase *et al*. [75], reported *in vitro* generation of NO by the reaction of H_2O_2 (10–50 mM) and L-arginine (10–20 mM) at pH 7.4 and 37 °C. Stöhr and Ullrich [76] observed that at acidic pH NO_2^- can dismutate to NO and nitrate. At acidic pH ascorbic acid can also reduce NO_2^- to yield NO and dehydroascorbic acid [77, 78]. Light-mediated reduction of NO_2^- to NO by carotenoids is another non-enzymatic mechanism [79]. Synthesis of NO by polyamines (PAs) is another important aspect of non-enzymatic synthesis. Tun *et al*. [80] found that PAs, spermidine and spermine increase NO release in *Arabidopsis* seedlings**.**

3. CONCLUSIONS

Since, the discovery of NO as signaling molecule, a huge wealth of information has been accumulated. The versatile NO occupies a significant position among signaling molecules, because of its multifaceted roles in different growth, physiological and biochemical events of plant life. The plant system does not have any single path of NO generation but possesses multiple NO generating systems of enzymatic and non-enzymatic origin. In spite of extensive research to find an animal equivalent of NOS in plants, the target molecule is still elusive and more work is required to unravel the mysterious NO.

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REFERENCES

[1] Culotta E, Koshland DJ. NO news is good news. Science 1992; 258: 1862-1865. http://dx.doi.org/10.1126/science.1361684

[2] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-376. http://dx.doi.org/10.1038/288373a0

[3] Ignarro LJ, BugaGM,Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Nat Acad Sci USA 1987; 84: 9265-9269. http://dx.doi.org/10.1073/pnas.84.24.9265

[4] Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-546. http://dx.doi.org/10.1038/327524a0

[5] Delledonne M, Xia YJ, Dixon RA, Lamb C. Nitric oxide functions as a signal in plant disease resistance. Nature 1998; 394: 585-588. http://dx.doi.org/10.1038/29087

[6] Khan MN, Siddiqui MH, Mohammad F, Naeem M. Interactive role of nitric oxide and calcium chloride in enhancing tolerance to salt stress. Nitric Oxide Biol Chem 2012; 27: 210-218. http://dx.doi.org/10.1016/j.niox.2012.07.005

[7] Neill SJ, Desikan R, Clarke A, Hancock, JT. Nitric oxide is a novel component of abscisic acid signalling in stomatal guard cells. Plant Physiol 2002; 128: 13-16. http://dx.doi.org/10.1104/pp.010707

[8] He YK, Tang RH, Hao Y, Stevens RD, Cook CW, Ahn SM, *et al.* Nitric oxide represses the *Arabidopsis* floral transition. Science 2004; 305: 1968-1971. http://dx.doi.org/10.1126/science.1098837

[9] Clarke D, Durner J, Navarre DA, Klessig DF. Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. Mol Plant Microbe Interact 2000; 13: 1380-1384. http://dx.doi.org/10.1094/MPMI.2000.13.12.1380

[10] Kumar D, Klessig DF. Differential induction of tobacco MAP kinases by the defense signals nitric oxide, salicylic acid, ethylene, and jasmonic acid. Mol Plant Mic. Interact 2000; 3: 347-351.

[11] Pagnussat GC, Lanteri ML, Lombardo MC, Lamattina L. Nitric oxide mediates the indole-acetic acid activation of a mitogen-activated protein kinase cascade involved in adventitious root formation. Plant Physiol 2004; 135: 279-286. http://dx.doi.org/10.1104/pp.103.038554

[12] Correa-Aragunde N, Lombardo C, Lamattina L. Nitric oxide: an active nitrogen molecule that modulates cellulose synthesis in tomato roots. New Phytol 2008; 79: 386-96.
http://dx.doi.org/10.1111/j.1469-8137.2008.02466.x

[13] Prado AM, Porterfield DM, FeijóJA. Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development 2004; 131: 2707-2714.
http://dx.doi.org/10.1242/dev.01153

[14] Bethke PC, Libourel IGL, Jones RL. Nitric oxide reduces seed dormancy in *Arabidopsis*. J Exp Bot 2006; 57: 517-526.
http://dx.doi.org/10.1093/jxb/erj060

[15] Beligni MV, Lamattina L. Nitric oxide stimulates seed germination andde-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 2000; 210: 215-221.
http://dx.doi.org/10.1007/PL00008128

[16] Klepper L. Nitric oxide (NO) and nitrogen dioxide (NO_2) emissions from herbicide-treated soybean plants. Atmos Environ 1979; 13: 537-542.
http://dx.doi.org/10.1016/0004-6981(79)90148-3

[17] Klepper L. Comparison between NO_x evolution mechanisms of wild-type and nr_1 mutant soybean leaves. Plant Physiol 1990; 93: 26-32.
http://dx.doi.org/10.1104/pp.93.1.26

[18] Cueto M, Hernández-Perera O, Martín R, Bentura ML, Rodrigo J, Lamas S, *et al.* Presence of nitric oxide synthase activity in roots and nodules of *Lupinus albus*. FEBS Lett 1996; 398: 159-164.
http://dx.doi.org/10.1016/S0014-5793(96)01232-X

[19] Butt YK, Lum JH, Lo SC. Proteomic identification of plant proteins probed by mammalian nitric oxide synthase antibodies. Planta 2003; 216: 762-771.18

[20] Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J 2001; 357: 593-615.
http://dx.doi.org/10.1042/0264-6021:3570593

[21] Ninnemann H, Maier J. Indications for the occurrence of nitric oxide synthases in fungi and plants, and the involvement in photoconidiation of *Neurospora crassa*. Photochem photobiol 1996; 64: 393-398.
http://dx.doi.org/10.1111/j.1751-1097.1996.tb02477.x

[22] Ribeiro Jr. EA, Cunha FQ, Tamashiro WMSC, Martins IS. Growth phase-dependent subcellular localization of nitric oxide synthase in maize cells. FEBS Lett 1999; 445: 283-286.
http://dx.doi.org/10.1016/S0014-5793(99)00138-6

[23] Wendehenne D, Pugin A, Klessig DF, Durner J. Nitric oxide: comparative synthesis and signalling in animal and plant cells. Trends Plant Sci 2001; 6: 177-183.
http://dx.doi.org/10.1016/S1360-1385(01)01893-3

[24] Barroso JB, Corpas FJ, Carreras A, Sandalio LM, Valderrama R, Palma JM, *et al.* Localization of nitric-oxide synthase in plant peroxisomes. J Biol Chem1999; 274: 36729-36733.
http://dx.doi.org/10.1074/jbc.274.51.36729

[25] del Río LA, Corpas FJ, Sandalio LM, Palma JM, Gómez M, Barroso JB. Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. J Exp Bot 2002; 53: 1255-1272.
http://dx.doi.org/10.1093/jexbot/53.372.1255

[26] Corpas FJ, Barroso JB, Carreras A, Valderrama R, Palma JM, Leon AM, *et al.* Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta 2006; 224: 246-254.
http://dx.doi.org/10.1007/s00425-005-0205-9

[27] Valderrama R, Corpas FJ, Carreras A, Fernández-Ocaña A, Chaki M, Luque F, Gómez-Rodríguez MV, Colmenero-Varea P, del Río LA, Barroso JB. Nitrosative stress in plants. FEBS Lett 2007; 581: 453-461.
http://dx.doi.org/10.1016/j.febslet.2007.01.006

[28] Guo FQ, Okamoto M, Crawford NM. Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science 2003; 302: 100-103.
http://dx.doi.org/10.1126/science.1086770

[29] Zeidler D, Zahringer U, Gerber I, Dubery I, Hartung T, Bors W, *et al.* Innate immunity in Arabidopsis thaliana: Lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc Natl Acad Sci USA 2004; 101: 15811-15816.
http://dx.doi.org/10.1073/pnas.0404536101

[30] Zhao MG, Tian QY, Zhang WH. Nitric oxide synthase-dependent nitric oxide production is associated with salt tolerance in Arabidopsis. Plant Physiol 2007; 144: 206-217.
http://dx.doi.org/10.1104/pp.107.096842

[31] Zemojtel T, Frohlich A, Palmieri MC, Kolanczyk M, Mikula I, Wyrwicz LS, *et al.* Plant nitric oxide synthase: a never-ending story? Trends Plant Sci 2006; 11: 524-525.
http://dx.doi.org/10.1016/j.tplants.2006.09.008

[32] Crawford NM, Galli M, Tischner R, Heimer YM, Okamoto M, Mack A. Response to Zemojtel, *et al.* Plant nitric oxide synthase: back to square one. Trends Plant Sci 2006; 11: 526-527.
http://dx.doi.org/10.1016/j.tplants.2006.09.007

[33] Kaiser WM, Weine H, Kanlbinder A, Tsai CB, Rockel P, Sonoda M*et al*.Modulation of nitrate reductase: some new insights, an unusual case and a potentially important side reaction. J Exp Bot 2002; 53: 875-882.
http://dx.doi.org/10.1093/jexbot/53.370.875

[34] Yamasaki H, Sakihama Y, Takahashi S. An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trends Plant Sci 1999; 4: 128-129.
http://dx.doi.org/10.1016/S1360-1385(99)01393-X

[35] Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G. Nitric oxide: the versatility of an extensive signal molecule. Annu Rev Plant Biol 2003; 54: 109-136.
http://dx.doi.org/10.1146/annurev.arplant.54.031902.134752

[36] Courtois C, Besson A, Dahan J, Bourque S, Dobrowolska G, Pugin A, Wendehenne D. Nitric oxide signalling in plants: interplays with Ca^{2+} and protein kinases. J Exp Bot 2008; 59: 155-163.
http://dx.doi.org/10.1093/jxb/erm197

[37] de la Haba P, Agüera E, Benítez L, Maldonado JM. Modulation of nitrate reductase activity in cucumber (*Cucumis sativus*) roots. Plant Sci 2001; 161: 231-237.
http://dx.doi.org/10.1016/S0168-9452(01)00328-4

[38] Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM. Regulation of nitric oxide (NO) production by plant nitrate reductase *in vivo* and *in vitro*. J Exp Bot 2002; 53: 103-110.
http://dx.doi.org/10.1093/jexbot/53.366.103

[39] Desikan R, Griffiths R, Hancock J, Neill S. A new role for an old enzyme: Nitrate reductas emediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Nat Acad Sci USA 2002; 99: 16314-16318.
http://dx.doi.org/10.1073/pnas.252461999

[40] Xu YC, Zhao BL. The main origin of endogenous NO in higher non-leguminous plants. Plant Physiol Biochem 2003; 41: 833-838.
http://dx.doi.org/10.1016/S0981-9428(03)00116-5

[41] Planchet E, Gupta KJ, Sonoda M, Kaiser WM. Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J 2005; 41: 732-743.
http://dx.doi.org/10.1111/j.1365-313X.2005.02335.x

[42] Sakihama Y, Nakamura S, Yamasaki H.Nitric oxide production mediated by nitrate reductase in the green alga *Chlamydomonas reinhardtii*: An alternative NO production pathway in photosynthetic organisms. Plant Cell Physiol 2002; 43: 290-297.
http://dx.doi.org/10.1093/pcp/pcf034

[43] Stöhr C, Strube F, Marx G, Ullrich WR, Rockel P. A plasma membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from nitrite*.* Planta 2001; 212: 835-841. http://dx.doi.org/10.1007/s004250000447

[44] Wildt J, Kley D, Rockel A, Rockel P, Segschneider HJ. Emission of NO from several higher plant species. J Geophys Res 1997; 102: 5919-5927. http://dx.doi.org/10.1029/96JD02968

[45] Mallick N, Rai LC, Mohn FH, Soeder CJ. Studies on nitric oxide (NO) formation by the green alga *Scenedesmus obliquus* and the diazotrophiccyanobacterium *Anabenadoliolum*. Chemosphere 1999; 39: 1601-1610. http://dx.doi.org/10.1016/S0045-6535(99)00058-2

[46] Dean JV, Harper JE. Nitric oxide and nitrous oxide production by soybean and winged bean during the *in vivo* nitrate reductase assay. Plant Physiol 1986; 82: 718-723. http://dx.doi.org/10.1104/pp.82.3.718

[47] Ji XB, Hollocher TC. Reduction of nitrite to nitric oxide by enteric bacteria. Biochem Biophys Res Commun 1988; 157: 106-108. http://dx.doi.org/10.1016/S0006-291X(88)80018-4

[48] Yamasaki H. Nitrite-dependent nitric oxide production pathway: implications for involvement of active nitrogen species in photo inhibition *in vivo*. Philos Trans R Soc Lond B Biol Sci 2000; 355: 1477-1488. http://dx.doi.org/10.1098/rstb.2000.0708

[49] Hao FS, Zhao SL, Dong H, Zhang H, Sun LR, Miao C. Nia1 and Nia2 are involved in exogenous salicylic acid-induced nitric oxide generation and stomata! closure in Arabidopsis. J Integr Plant Biol 2010; 52: 298-307. http://dx.doi.org/10.1111/j.1744-7909.2010.00920.x

[50] Zhao MG, Chen L, Zhang LL, Zhang WH. Nitric Reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in Arabidopsis. Plant Physiol 2009; 151: 755-767. http://dx.doi.org/10.1104/pp.109.140996

[51] Oliveira HC, Saviani EE, Oliveira JFP, Salgado I. Nitrate reductase-dependent nitric oxide synthesis in the defense response of *Arabidopsis thaliana* against *Pseudomonas syringae*. Trop Plant Pathol 2010; 35: 104-107. http://dx.doi.org/10.1590/S1982-56762010000200005

[52] Seligman K, Saviani EE, Oliveira HC, Pinto-Maglio CA, Salgado I. Floral transition and nitric oxide emission during flower development in *Arabidopsis thaliana* is affected in nitrate reductase-deficient plants. Plant Cell Physiol 2008; 49: 1112-1121. http://dx.doi.org/10.1093/pcp/pcn089

[53] Corpas FJ, Carreras A, Esteban FJ, Chaki M, Valderrama R, del Rio LA, *et al.* Localization of S-nitrosothiols and assay of nitric oxide synthase and S-nitrosoglutathione reductase activity in plants. Methods Enzymol 2008; 437: 561-574. http://dx.doi.org/10.1016/S0076-6879(07)37028-6

[54] Palma JM, Sandalio LM, Corpas FJ, Romero-Puertas MC, McCarthy I, del Río LA. Plant proteases, protein degradation, and oxidative stress: role of peroxisomes. Plant Physiol Biochem 2002; 40: 521-530. http://dx.doi.org/10.1016/S0981-9428(02)01404-3

[55] Zrenner R, Stitt M, Sonnewald U, Boldt R. Pyrimidine and purine biosynthesis and degradation in plants. Annu Rev Plant Biol 2006; 57: 805-836. http://dx.doi.org/10.1146/annurev.arplant.57.032905.105421

[56] Tischner R, Planchet E, Kaiser WM. Mitochondrial electron transport as a source for nitric oxide in the unicellular green alga *Chlorella sorokiniana*. FEBS Lett 2004; 576: 151-155. http://dx.doi.org/10.1016/j.febslet.2004.09.004

[57] Planchet E, Jagadis Gupta K, Sonoda M, Kaiser WM. Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J 2005; 41: 732-743. http://dx.doi.org/10.1111/j.1365-313X.2005.02335.x

[58] Gupta KJ, Stoimenova M, Kaiser WM. In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, *in vitro* and *in situ*. J Exp Bot 2005; 56: 2601-2609. http://dx.doi.org/10.1093/jxb/eri252

[59] Foresi N, Correa-Aragunde N, Parisi G, Caló G, Salerno G, Lamattina L. Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent. Plant Cell 2010; 22: 3816-3830. http://dx.doi.org/10.1105/tpc.109.073510

[60] Derelle E, Ferraz C, Rombauts S, Rouzé P, Worden AZ, Robbens S, *et al.* Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. Proc Natl Acad Sci USA 2006; 103: 11647-11652. http://dx.doi.org/10.1073/pnas.0604795103

[61] Trapido-Rosenthal HG, Sharp KH, Galloway TS, Morall CE. Nitric oxide and cnidarian-dinoflagellate symbioses: pieces of a puzzle. Am Zool 2001; 41: 247-257. http://dx.doi.org/10.1668/0003-1569(2001)041[0247:NOACDS]2.0.CO;2

[62] Trapido-Rosenthal HG, Zielke S, Owen R, Buxton L, Boeing B, Bhagooli R, *et al.* Increased zooxanthellae nitric oxide synthase activity is associated with coral bleaching. Biol Bull 2005; 208: 3-6. http://dx.doi.org/10.2307/3593094

[63] Kim D, Yamaguchi K, Oda T. Nitric oxide synthase-like enzyme mediated nitric oxide generation by harmful red tide phytoplankton, *Chatoniella marina*. J Plankton Res 2006; 28: 613-620. http://dx.doi.org/10.1093/plankt/fbi145

[64] Gupta KJ, Igamberdiev AU, Kaiser WM. New insights into the mitochondrial nitric oxide production pathways. Plant Sig Behav 2010; 5: 999-1001.

[65] van den Bosch H, Schutgens RB, Wanders RJ, Tager JM. Biochemistry of peroxisomes. Annu Rev Biochem 1992; 61: 157-197. http://dx.doi.org/10.1146/annurev.bi.61.070192.001105

[66] Mullen RT, RN Trelease. Biogenesis and membrane properties of peroxisomes: does the boundary membrane serve and protect? Trends Plant Sci 1996; 1: 389-394.

[67] Barroso JB, Corpas FJ, Carreras A, Sandalio LM, Valderrama R, Palma JM, *et al.* Localization of nitric oxide synthase in plant peroxisomes. J Biol Chem 1999; 274: 36729-36733. http://dx.doi.org/10.1074/jbc.274.51.36729

[68] Corpas FJ, Barroso JB, León AM, Carreras A, Quirós M, Palma JM, *et al.* Peroxisomes as a source of nitric oxide. In Magalhaes JR, Singh RP, Passos LP, eds, Nitric oxide signaling in higher plants. Studium Press, Houston, 2004; 111-129.

[69] Corpas FL, Hayashi M, Mano S, Nishimura M, Barroso JB. Peroxisomes are required for *in vivo* nitric oxide accumulation in the cytosol following salinity stress of Arabidopsis plants. Plant Physiol 2009; 151: 2083-2094. http://dx.doi.org/10.1104/pp.109.146100

[70] Boucher JL, Genet A, Vadon S, Delaforge M, Mansuy D. Formation of nitrogen oxides and citrulline upon oxidation of N^{w}-hydroxy-l-arginine by hemeproteins. Biochem Biophys Res Commun 1992a; 184: 1158-1164. http://dx.doi.org/10.1016/S0006-291X(05)80004-X

[71] Huang J, Sommers EM, Kim-Shapiro DB, King SB. Horseradish peroxidase catalyzed nitric oxide formation from hydroxyurea. J Am Chem Soc 2002; 124: 3473-3480. http://dx.doi.org/10.1021/ja012271v

[72] Sturms R, DiSpirito AA, Hargrove MS. Plant and cyanobacterial hemoglobins reduce nitrite to nitric oxide

under anoxic conditions. Biochem 2011; 50: 3873-3878. http://dx.doi.org/10.1021/bi2004312

[73] Boucher JL, Genet A, Valdon S, Delaforge M, Henry Y, Mansuy D. Cytochrome P450 catalyzes the oxidation of Nw-hydroxy-l-arginine by NADPH and O_2 to nitric oxide and citrulline. Biochem Biophys Res Commun 1992b; 187: 880-886. http://dx.doi.org/10.1016/0006-291X(92)91279-Y

[74] Mansuy D, Boucher JL. Oxidation of N-hydroxyguanidines by cytochromes P450 and NO-synthases and formation of nitric oxide. Drug Metab Rev 2002; 34: 593-606. http://dx.doi.org/10.1081/DMR-120005661

[75] Nagase S, Takemura K, Ueda A, Hirayama A. A novel nonenzymatic pathway for the generation of nitric oxide by the reaction of hydrogen peroxide and d- or l-arginine. FEBS Lett 1997; 233: 150-153.

[76] Stöhr C, Ullrich WR, Generation and possible roles of NO in plant roots and their apoplastic space. J Exp Bot 2002; 53: 2293- 2303. http://dx.doi.org/10.1093/jxb/erf110

[77] Henry YA, Ducastel B, Guissani A. Basic chemistry of nitric oxide and related nitrogen oxides. In: Henry YA, Guissani A, Ducastel B, Eds. Nitric Oxide Research from Chemistry to Biology. Austin, USA: Landes Co Biomed Publ 1997; pp. 15-46. http://dx.doi.org/10.1007/978-1-4613-1185-0_3

[78] Beligni MV, Fath A, Bethke PC, Lamattina L, Jones, RL. Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. Plant Physiol 2002; 129: 1642-1650. http://dx.doi.org/10.1104/pp.002337

[79] Cooney RV, Harwood PJ, Custer LJ, Franke AA. Light-mediated conversion of nitrogen dioxide to nitric oxide by carotenoids. Environ Health Persp 1994; 102: 460-462. http://dx.doi.org/10.1289/ehp.94102460

[80] Tun NN, Santa-Catarina C, Begum T, Silveira V, Handrow F, Lohe I, *et al.* Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 2006; 47: 346-354. http://dx.doi.org/10.1093/pcp/pci252

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