

Interplay of Nitric Oxide and Hydrogen Peroxide in Root Development

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Suhel M et al. Reactive Oxygen Species 9(25):1–12, 2020; ©2020 Cell Med Press
<http://dx.doi.org/10.20455/ros.2020.801>

(Received: July 1, 2019; Revised: August 2, 2019; Accepted: August 3, 2019)

ABSTRACT | Among various signaling molecules, nitric oxide (NO) and hydrogen peroxide (H₂O₂) play a crucial role in plant developmental process such as root development. It is influenced by endogenous levels of signaling molecules like NO and H₂O₂, by controlling the polarity of cell, the pattern of cell division and cell cycle dynamics. The NO and H₂O₂ also regulate the cell functions either by up-regulating or down-regulating several genes which govern different physiological functions. These two signaling molecules (NO and H₂O₂) also interact with various plant hormones and guide the peculiar function which is implicated in the process of root development. Recent past studies have demonstrated the crosstalk of NO and H₂O₂ in root development under stressed as well as non-stressed conditions. In this review, implication of NO and H₂O₂ crosstalk and its relation with other signaling molecules and phytohormones in root development is discussed.

KEYWORDS | Abiotic stress; Hydrogen peroxide; Nitric oxide; Phytohormones; Polar transport; Polarity; Root development

ABBREVIATIONS | APP1, Arabidopsis p-loop NTPase; IAA, indole-3-acetic acid; MPK, mitogen-activated protein kinase; NO, nitric oxide; NOS, nitric oxide synthase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNP, sodium nitroprusside; WOX5, WUSCHEL-related homeobox 5

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1. INTRODUCTION

Growth of root facilitates plants to reach out to their essential nutrients which are present in greater depth and larger areas. Besides this, roots also provide anchorage to plants. Based on ontogeny, roots are of several types such as primary, lateral, and adventitious roots, and their ontogenesis is regulated by various intrinsic and extrinsic signals which are required for their proper growth and development [1]. Root development is considered as a crucial phenomenon which solely determines the survival of the plant [1]. In the formation of adventitious roots, development of a meristematic tissue occurs after removal of the primary root system. In this process, the plant hormone auxin (indole-3-acetic acid or IAA) acts as a regulatory factor which promotes de-differentiation in order to re-establish the new apical meristem [2].

Recent studies reveal that reactive nitrogen species (RNS) and reactive oxygen species (ROS) play a significant role in plant growth and development by modulating endogenous signals. In the last decade, it has been demonstrated that nitric oxide (NO), an RNS, plays a crucial role during development of plants [1, 3–5]. NO, as a signaling molecule, is mainly concerned with germination of seeds, alleviation of seed dormancy, stomatal closure, fruit maturation, senescence, iron-metabolism, and floral transition [6]. In addition, it also plays a crucial role in shaping root architecture and its growth [7]. Several other biochemical and molecular studies have also recognized implication of NO and its derived molecules in root development [1, 8]. Moreover, NO is involved in establishing the interactions between pulse crops root and rhizobia for converting nitrogen gas into ammonia by involving a complex signal cascade [9]. In roots, NO production depends on conditions which prevail under specific adverse conditions and may differ according to the age of plants, stress intensity, and exposition time [3, 10]. In diverse plant responses, it has been demonstrated that NO and auxin are involved in the development of primary root [6] as well as in the development of adventitious root [11]. It has been reported that NO influences and lowers IAA oxidase activity in *Medicago truncatula* growing under cadmium stress thereby showing elevated level of IAA [12]. Evidence suggests that NO acts downstream of auxin action in plants and is involved in primary and lateral root formation [7, 13]. Cytokinins are also considered as key hormones that

regulate the development of root and its vascular differentiation and root gravitropism, and NO acts downstream of cytokinins [14].

ROS (e.g., H_2O_2) produced during a stress condition can either cause oxidative damage or stimulate signal transduction [15, 16], but the mechanism involved in this pathway is still unsolved. It has been demonstrated that like NO, H_2O_2 also plays an essential role in various physiological and developmental processes such as growth of root and its development [17, 18]. The H_2O_2 may affect different parts of plants by regulating either homeostasis or relative gene expression [19]. The crosstalk between NO and H_2O_2 has been considered to be an essential factor in influencing plant developmental processes such as root growth and development [17, 18]. In view of the above, this review discusses the possible roles of NO and H_2O_2 , and their crosstalk in root development.

2. NITRIC OXIDE AND H_2O_2 PRODUCTION IN PLANTS

Nitric oxide is a second messenger and participates in different biochemical activities in the course of plant development and assimilation of nutrients. Substantial evidence suggests that growth and development of root system is affected by NO [10, 13, 20–26]. Nitric oxide synthase (NOS) and nitrate reductase are involved in NO generation [1]. Although NOS is not yet reported in plants [27, 28], experiments using an NOS inhibitor showed certain evidence of existence of L-arginine pathway-dependent production of NO [29]. Accumulation of NO revealed by using 4,5-diaminofluorescein diacetate (DAF-2DA) in hypocotyls, root, and seeds further supports existence of NOS in plants [20, 23, 30, 31]. In the root system, NO generation is noticed in statocytes of root cap, quiescent center, and distal portion of root meristem [23]. The most eminent production site of NO is the distal portion in the transition zone of root [30, 31].

NO production involves two pathways in plant tissues, i.e., the enzymatic and the non-enzymatic pathways [32, 33]. Nitrate reductase, the first identified NO biosynthetic enzyme [34], catalyzes the reduction of nitrate to nitrite, and it is also able to reduce nitrite to NO using NADPH [35]. In plants, evidence for NOS-like activity is indicated by the existence of *Oryza sativa* nitric oxide associated pro-

tein 1 (OsNOA1/RIF1; NO synthase 1; later renamed AtNOA1) in maintaining chloroplast translation [36–38]. The AtNOA1 plays a pivotal role in temperature stress acclimation, rubisco formation, and chlorophyll biosynthesis in rice [39]. Furthermore, in *Arabidopsis thaliana*, AtNOA1 has shown to be involved in salicylic acid-mediated root waving through Ca^{2+} signal transduction pathway [40]. Recently, OsNOA1 has been shown to be a crucial component in regulating the pattern of chloroplast proteins under low temperature stress. In this connection, studies by several investigators [41] have characterized NOS in the plant kingdom and suggested that NOS governed the generation of NO in green alga *Ostreococcus tauri*. These studies provide evidence for the existence of NOS-like activity in plants. Besides this, the evidence for existence of L-arginine-dependent NOS activity in plants has been reported [42]. The same research group also reported the localization of NOS in peroxisomes of pea seedlings [43]. Xanthine oxidase/dehydrogenase and cytochrome P450 systems are among the other important factors which have occasionally been suggested as sources of endogenous NO in plants [44]. In the non-enzymatic pathway, NO is produced from the reduction of nitrite at low pH in the apoplast of the aleurone cell layer during seed germination [45]. NO production is essential for the various biochemical tasks throughout the growth and development of plants.

In plants, aerobic metabolism results in the formation of H_2O_2 as a byproduct [46]. H_2O_2 is formed via various pathways such as the electron transport chain, photorespiration, and redox reactions in peroxisomes, chloroplasts, and mitochondria [19]. Several findings prove that synthesis of H_2O_2 occurs via several enzymatic systems, such as cell wall peroxidase [47], amine oxidase, oxalate oxidase [48], and flavin-enzymes [49]. The cellular level of H_2O_2 may also be increased by the action of NADPH oxidases which generate superoxide [50–52]. Superoxide is converted into H_2O_2 with the help of the enzyme superoxide dismutase (SOD) [50–52]. NADPH oxidase-derived superoxide and H_2O_2 also occur in *Arabidopsis thaliana* under salt stress [53]. Besides this, few other oxidases are also involved in the production of H_2O_2 by oxidizing their own substrates, such as oxidases of glucose and glycolate [54] and sulfite [55]. Recently, a number of studies have suggested the involvement of H_2O_2 signaling in plant

developmental processes, such as root development [19, 56–60].

3. NITRIC OXIDE AND ROOT DEVELOPMENT

Growth and development of root results from a mutual interaction of NO with other plant hormones (e.g., auxin, ethylene, abscisic acid) and reactive oxygen species [61]. Root branching occurs via emergence of lateral roots from pericycle, an inner tissue of mature root. Formation of lateral root process includes initialization of lateral root primordia and their emergence [62]. Lateral primordium emerges from pericycle by passing through endodermis, cortical, and epidermal tissues [63]. Studies showed that lateral and adventitious root formation and maturation are stimulated by NO [64, 65].

Shen et al. [66] showed that NO regulated the expression of CYCD3;1 that complemented *Arabidopsis thaliana* NO-deficient mutant *Atnos/noa1* through cytokinin and thus maintained meristematic activity in root and shoot. In another study, expression of WUSCHEL-related homeobox 5 (WOX5) gene was responsible for undifferentiated status of root stem cells in quiescent center while NOS inhibition led to reduced expression of WOX5 and this effect was reversed by sodium nitroprusside (SNP) [31] (Figure 1). Under nitrate supply, NO modulates formation and growth of root system [67–70]. Production of NO is related with NO_3^- supply which further regulates the root development [67–69]. In plants, several studies showed that NO responds in two ways. Firstly, NO modulates the root growth in the presence of NO_3^- [67, 69]. Manoli et al. [67] have suggested that NO signaling pathway and NO_3^- concentration are interlinked during increase of root length. NO also induces lateral root formation by partial nitrate nutrition (PNN) in rice. Secondly, NO regulates the nitrogen (N) uptake by inducing the n-transporter gene expression under PNN [69].

NO is not only involved in root growth promotion but also responsible for root growth decline. *Arabidopsis* mutant hypersensitive to NO showed under-expression of chlorophyll *a/b* binding protein (*nox1/cue1*), thus exhibiting relation between NO and root length [23]. In *Arabidopsis*, it is observed that a low level of NO causes programmed cell death, DNA damage, and cell cycle arrest at G1 and G2/M

phases resulting in reduction of root growth [71] (Figure 1).

4. NITRIC OXIDE AND ITS INTERACTION WITH PLANT HORMONES DURING ROOT DEVELOPMENT

There are reports about a close relation between NO and auxin in the regulation of root growth and development [23, 72, 73]. NO acts downstream of auxin and regulates lateral root formation but it inhibits the elongation, by diminishing auxin level in root under iron (Fe) deficiency in rice [7]. However, it has been suggested that response of root apex mediated by NO under NO₃⁻ supply is regulated by auxin in maize [74]. On the other hand, a high level of NO retards basipetal transport of auxin in *Arabidopsis* root by diminishing the PIN1 abundance [23] (Figure 1). NO is reported to induce lateral root formation in rice by involving heme oxygenase and the effect of NO is reversed by 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO, a specific NO scavenger) [75]. Correa-Aragunde et al. [20] reported that NO, localized in lateral roots primordia, was able to reduce lateral root formation in tomato by involving auxin-dependent signal transduction pathway. It has been noticed that peroxisome-derived NO and IAA are promoted by indole-3-butyric acid, which are collectively required for lateral root formation in *Zea mays* L. [76]. Auxin-mediated NO production was shown to be involved in lateral root formation by involving auxin transport-reduced phototropin1 (Phot1) [77]. In *Medicago* roots, NO resists oxidative damage by reducing ROS accumulation under cadmium stress which boosts IAA accumulation by decreasing its degradation via IAA oxidase activity [12, 78]. Moreover, a synergistic role of NO and auxin was also elaborated by Freschi [6] in the regulation of various plant responses including root growth. Recently, nitrate-mediated production of NO by nitrate reductase has been shown to promote lateral root formation and elongation of seminal roots by regulating auxin distribution [8].

Cytokinins have also been shown to be involved in modulation of root development and cell differentiation. Reduced sensitivity and retardation of root growth is distinguished in NO-deficient mutant *noal*. Further, it has been shown that addition of cytokinins

caused activation of CYCD3;1, modulated by NO, during proliferation of cells; defects in *noal* mutant in root tissue can be complemented by the overexpression of CYCD3;1 [66]. Contrarily, NO-treated plant displays decreased cytokinins activity, and the primary root growth is inhibited by exogenously applied cytokinins. A possible mechanism for this modulation may be the interaction between NO and cytokinins because a feasible reaction can occur between peroxynitrite and zeatin as reported by Liu et al. [79]. Cytokinin signaling is repressed by NO by inhibiting the phosphorylation activity via S-nitrosylation of the histidine phosphotransfer protein [80].

5. HYDROGEN PEROXIDE AND ROOT DEVELOPMENT

Root is one of the most important organs of plant system, which is not only a support system but also for the absorption of mineral nutrients and water, additionally sensing to the immediate environmental changes. Root growth depends on the cell growth and its differentiation in root tip [81]. ROS such as H₂O₂ maintain balance between the cell growth and cell division in plants. ROS distribute within the tip of root, especially in the elongation zone [82, 83]. A protein located into the mitochondria of root meristem of *Arabidopsis* called *Arabidopsis* p-loop NTPase (APP1) shows ATPase activity and hydrolyzes nucleoside triphosphate. Stem cell differentiation is observed in root due to mutation in APP1 gene which causes reduction in the ROS level and induced cell division in the quiescent center [84]. In addition to this, ROS also play a key role in determining the elongation zone by regulating the cell cycle. In this way, ROS are well established as cell cycle regulators in animal system [85].

ROS including H₂O₂ also play a key role in the formation of lateral roots [86]. Cells representing lateral root primordia specifically express SKP2Bp:GFP (for S-Phase Kinase-Associated Protein 2B) genes [86, 87]. During lateral root development, lateral root initiating cell, which is called founder cell, expresses SKP2B [86]. UP BEAT1 (UPB1), a transcription factor, represses the expression of class III peroxidase and regulates ROS homeostasis in root tip cells [82]. Manzano et al. found that ROS are accumulated in the UPB1-expressing

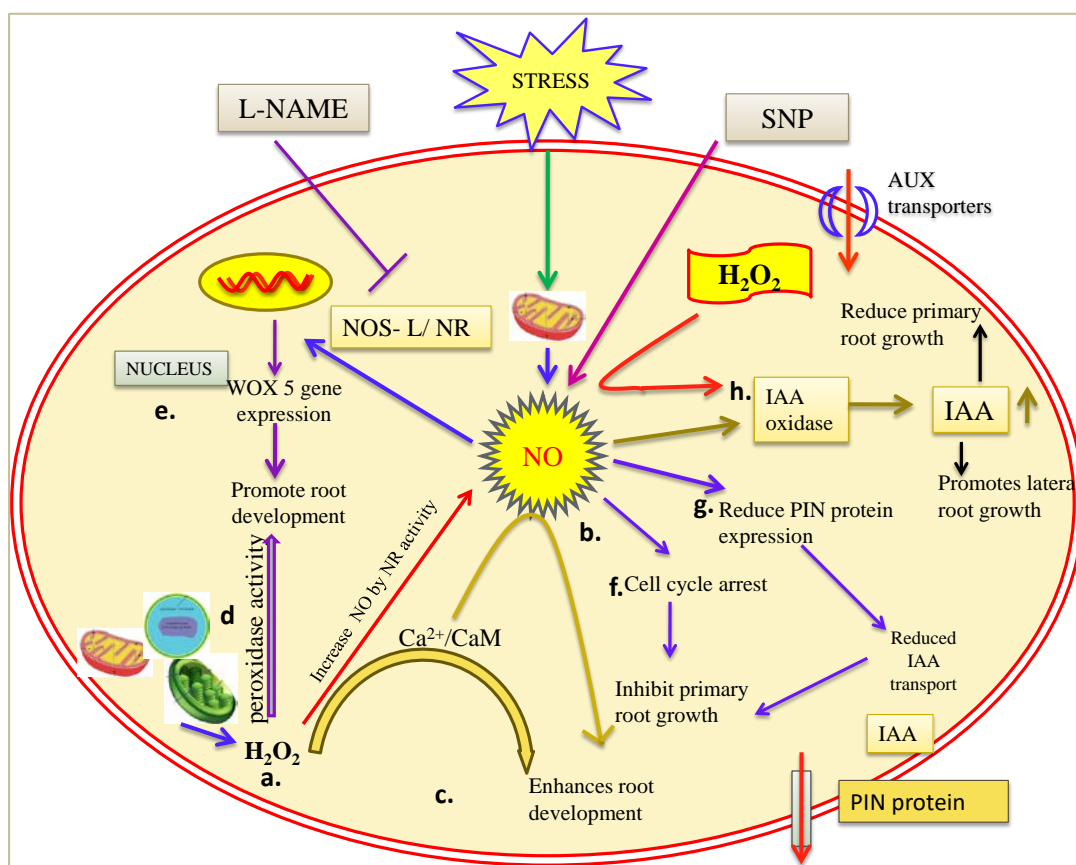


FIGURE 1. Production sites of H₂O₂ (ROS) and NO in cells and their role in the regulation of root development by interacting with other molecules. (a) H₂O₂ is produced in the cell during electron transport chain and redox reactions in the chloroplasts, mitochondria, and peroxisomes; (b) Level of NO is increased in the cell by nitric oxide synthase (NOS) and nitrate reductase (NR) activity which are enhanced by H₂O₂, and NO is also accumulated in the cell by non-enzymatic pathways in response to environmental stress; (c) Ca²⁺/CaM by interacting with H₂O₂ and NO enhances development of root; (d) The enzyme peroxidase promotes root development by decreasing the level of H₂O₂ in the cell; (e) WOX5 gene expression is reduced by NOS inhibitor (L-NAME) while its expression is reversed by sodium nitroprusside (SNP); (f) Cell cycle is arrested at G1/G2M phase at low level of NO in *Arabidopsis*; (g) NO reduces the primary root growth by inhibiting the expression of PIN protein; and (h) NO and H₂O₂ together promote primary root growth by up-regulating the activity of IAA-oxidase while lateral root growth is stimulated by NO which down-regulates IAA oxidase activity.

pericycle cells in lateral root primordial stage. Additionally, later stage of lateral root primordia accumulates ROS during lateral root development [87]. On the other hand, it has been demonstrated that UPB1 is also responsible for regulating the ROS level during iron deficiency which reduces the lateral root emergence [88]. These findings suggest that UPB1

regulates the accumulation of ROS which are required for lateral root emergence. In addition, Li et al. [89] reported that lateral root formation is negatively regulated by Atrboh D and F (NADPH oxidase proteins in *Arabidopsis*). In this study, peroxidase activity was reported higher in double mutant of Atrboh D and F than wild type together with an increase in lat-

eral root density. Connection of ROS with auxin has also been reported which is required for lateral root initiation and its development [90]. Further studies showed that development of lateral roots in *Atrboh D/F* mutants was inhibited by treatment with NPA (an auxin transport inhibitor) and co-treatment with auxin increased the number of lateral root primordia observed similarly in wild type [89]. The studies have shown that ROS may regulate lateral root emergence in an auxin-independent manner [89, 90]. Contrary to above studies, exogenously applied H_2O_2 repressed the cell cycle regulatory gene which decreased meristem size of root [91].

6. CROSSTALK OF NO AND H_2O_2 IN ROOT DEVELOPMENT

Substantial evidence suggests that NO is an essential signaling molecule involved in diverse physiological processes including growth of root and its development [18, 40, 92]. Hydrogen peroxide serves as a signal that enhances the production of NO endogenously. Lin et al. [93] reported that production of NO may be stimulated by H_2O_2 via increasing the activity of nitrate reductase in leaves of *noe1* mutant under high intensity light. A possible mechanism might involve the role of H_2O_2 and NO synergistically which stimulate root development by enhancing the functions of indole-acetic acid oxidase (IAAO) and polyphenol oxidase (PPO) in *Chrysanthemum* [17]. Interconnection of H_2O_2 , NO, and Ca^{2+} signaling pathways along with auxin is reported to be involved in inducing development of adventitious roots [94] (Figure 1).

In plants, mitogen-activated protein kinases (MPKs) act as prime signaling molecules which are triggered under certain environmental and developmental states [95, 96]. Previously, it has been demonstrated that MPK6 inflects growth and development of plant by interacting with NO and H_2O_2 [96]. MPK6 also controls H_2O_2 -stimulated growth of root by modulating the Ca^{2+} carrier in *Arabidopsis* [96]. In *Arabidopsis*, NO promotes programmed cell death and is required for managing cadmium stress, by boosting MPK6-mediated signaling [97]. Further, under zinc stress interplay of H_2O_2 and NO participates in the up-regulation of root growth via promoting antioxidant system, reducing the lipid peroxidation, and enhancing gene expression [98]. A

study also showed that combined action of H_2O_2 and NO is down-regulated by Ca^{2+} [94]. The role of H_2O_2 and NO during salt stress is discussed elsewhere [99–101]. In *Tagetes erecta*, H_2O_2 mediates increase in root length under abiotic stress [102] and under salt stress, it alleviates mesophyll damage participating with NO [103]. In *Arabidopsis thaliana*, NO is involved in influencing lateral root development along with *N*-isobutyl decanamide [104]. A positive relationship has been reported among H_2O_2 , NO, and Ca^{2+} /CaM signaling during formation of adventitious roots [64]. Thus, the interaction between H_2O_2 and NO can trigger various responses in plants. In previous studies, it is clearly observed that exogenously applied NO and H_2O_2 may induce the formation of roots but a clear mechanism for this physiological response is still unknown. A schematic model showing crosstalk of NO and ROS and its implications in root development is depicted in Figure 1.

7. CONCLUSION AND FUTURE PROSPECTS

NO and H_2O_2 in association with phytohormones like auxins are considered as important components governing root development. The sources of NO and H_2O_2 formation inside the cells involve several enzyme complexes and are considered as a very crucial phenomenon. NO- and H_2O_2 -induced alterations in endogenous signals and gene expression have helped much in understanding the complex physio-chemical and molecular networks which are involved in root development. However, there are still many missing links such as how levels of endogenous chemicals and gene expression control and how this information is translated into final plant response such as root development.

ACKNOWLEDGMENTS

M.S. and T.H. contributed equally to the work. M.S., T.H., A.F., A.P., and S.K.S. are thankful to the University Grants Commission, New Delhi for providing D.Phil. Scholarship. V.P.S. is grateful to the Department of Biotechnology, New Delhi (BT/PR12980/BPA/118/80/2015) for providing financial assistance. S.S. and S.M.P. are thankful to the SERB-DST, New Delhi (EMR/2016/004745) for providing financial assistance. The authors declare no conflicts of interest.

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