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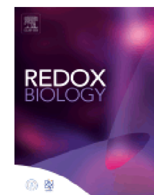

Review article

# Plant peroxisomes: A nitro-oxidative cocktail

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ABSTRACT

Although peroxisomes are very simple organelles, research on different species has provided us with an understanding of their importance in terms of cell viability. In addition to the significant role played by plant peroxisomes in the metabolism of reactive oxygen species (ROS), data gathered over the last two decades show that these organelles are an endogenous source of nitric oxide (NO) and related molecules called reactive nitrogen species (RNS). Molecules such as NO and $H_2O_2$ act as retrograde signals among the different cellular compartments, thus facilitating integral cellular adaptation to physiological and environmental changes. However, under nitro-oxidative conditions, part of this network can be overloaded, possibly leading to cellular damage and even cell death. This review aims to update our knowledge of the ROS/RNS metabolism, whose important role in plant peroxisomes is still underestimated. However, this pioneering approach, in which key elements such as β-oxidation, superoxide dismutase (SOD) and NO have been mainly described in relation to plant peroxisomes, could also be used to explore peroxisomes from other organisms.

## 1. Introduction

In morphological terms, peroxisomes are very simple organelles composed of a dense matrix surrounded by a single membrane. Antioxidant catalase and $H_2O_2$-producing flavin oxidases are essential enzymatic components of these organelles. However, it is surprising to note that their biochemical composition can change depending on the organism, organ type, development stage and environment involved [25,44,53,88,97,106,112,113].

In higher plants, peroxisomes possess extraordinary metabolic plasticity because they house wide range of pathways such as fatty acid β-oxidation, glyoxylate cycles, photorespiration, purine catabolism, plant hormone biosynthesis (indole-3-acetic acid and jasmonic acid) and polyamine catabolism [20,54,63,65,85,88,108]. Many of these pathways involve other subcellular compartments, suggesting that peroxisomes must have a pertinent retrograde signaling among the different subcellular compartments which must integrate their functions under optimal physiological conditions or trigger appropriate responses in unforeseen adverse situations [55].

In some cases, these metabolic interactions may involve dynamic and reversible functional and morphological adaptations between peroxisomes and other organelles such as chloroplasts and oil bodies during physiological processes such as photorespiration [19,51,84] and seed germination [35] which enable metabolic exchanges between the organelles to be optimized [63]. So far, over 300 plant peroxisomal proteins have been identified [69,93]; however, innovative approaches using proteomic and bioinformatic technologies, with the combination of highly purified peroxisomes from different plant tissues at different developmental stages, have brought to light new and unexpected components with hitherto unknown functions [3,47,60,62,71,88,99]. Another intriguing aspect of plant peroxisomes is their generation and division and the number of proteins from the matrix and membrane imported and integrated into these organelles through processes involving two major pathways: the peroxisomal targeting signal 1 (PTS1) located at the three final amino acids on the C-terminus and the PTS2 located within the first 30–40 amino acids at the amino-terminal end. This involves cleaving PTS2 and the collaboration of a battery of proteins called peroxins (PEXs) which are peroxisomal biogenesis factors encoded by *PEX* genes involved in matrix protein transport through PTS1 and PTS2 pathways. They are located in both the cytosol and peroxisomal membrane [34].

This review focuses on the latest advances in relation to the metabolism of ROS and RNS in plant peroxisomes and its functional implications, suggesting that these organelles constitute a nitro-oxidative

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**Table 1.**
Plant peroxisomal enzymes involved in ROS and RNS metabolism.

| Pathway/enzyme name | Intraperoxisomal localization | ROS/RNS involved |
|---|---|---|
| **RNS-generating enzymes** | | |
| L-arginine-dependent nitric oxide synthase (NOS) | Matrix | $^{\cdot}NO$ |
| S-Nitrosoglutathione reductase (GSNOR) | Matrix | GSNO |
| **ROS-generating enzymes** | | |
| ***-Fatty acid degradation*** | | |
| Short chain acyl-CoA oxidase | Matrix | $H_2O_2$ |
| Medium chain acyl-CoA oxidase | Matrix | $H_2O_2$ |
| Long chain acyl-CoA oxidase | Matrix | $H_2O_2$ |
| ***- Photorespiration*** | | |
| Glycolate oxidase | Matrix | $H_2O_2$ |
| ***-Purine metabolism*** | | |
| Urate oxidase (UO) | Matrix | $H_2O_2$ |
| Xanthine oxidoreductase (XOR) | Matrix | $O_2^{\cdot-}$ |
| ***-Superoxide-generating membrane polypeptides (PMPs)*** | Membrane-bound | $H_2O_2$, $O_2^{\cdot-}$ |
| **Others** | | |
| Sarcosine oxidase (SOX) | Matrix | $H_2O_2$ |
| Sulphite oxidase (SO) | Matrix | $H_2O_2$ |
| Polyamine oxidase (PAO) | Matrix | $H_2O_2$ |
| **Antioxidant enzymes** | | |
| **Catalase** | Matrix | $H_2O_2$ |
| **Superoxide dismutase (SOD)** | | |
| Mn-SOD | Matrix & Membrane-bound | $O_2^{\cdot-}$ |
| CuZn-SOD | Matrix | $O_2\cdot-$ |
| Fe-SOD | ? | |
| **Ascorbate-glutathione cycle** | | |
| Ascorbate peroxidase (APX) | Membrane-bound | $H_2O_2$ |
| Glutahtione reductase (GR) | Matrix | |
| Monodehydroascorbate reductase (MDAR) | Matrix & Membrane-bound | |
| Dehydroascorbate reductase (DHAR) | Matrix | |
| **Peroxiredoxin-like protein** | Matrix | $H_2O_2$, ROOH |
| **NADPH-regenerating systems** | | |
| Glucosa–6-phosphate dehydrogenase (G6PDH) | Matrix | |
| 6-phosphogluconate dehydrogenase (6PGDH) | Matrix | |
| NADP-isocitrate dehydrogenase (NADP-ICDH) | Matrix | |

'cocktail' within the cell. We will also discuss some advances made with regard to the plant peroxisomal metabolism which have increased our knowledge of the peroxisomes of other organisms including yeast and mammals.

## 2. Plant peroxisomal components responsible for ROS and RNS metabolisms

Table 1 summarizes the principal enzymes in plant peroxisomes involved in the generation of ROS/RNS as well as antioxidant enzymes localized in both the matrix and membrane which regulate these molecules. The data indicate that plant peroxisomes have a nitro-oxidative metabolism with complex regulatory antioxidative machinery.

The term reactive oxygen species (ROS) includes molecules such as superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$), hydroperoxyl radicals ($HO_2^{\cdot}$), alkoxyl radicals ($RO^{\cdot}$), peroxyl radicals ($ROO^{\cdot}$) and non-radical $O_2$ derivatives such as hydrogen peroxide ($H_2O_2$). Superoxide radicals and hydrogen peroxide are two of the most studied ROS in plants. ROS are generated under physiological conditions and plants containing a complex battery of antioxidant defence systems to regulate their production [4]. Interestingly, peroxisomes are important sites for the generation of these ROS, mainly via photorespiration and fatty acid β-oxidation pathways [25,32,40,42,83]. Thus, peroxisomes as cell compartments, with high rates of $H_2O_2$ production, play an important role in defence mechanisms and ROS-mediated cross-talk between cell compartments. The principal ROS regulatory enzymatic systems in plant peroxisomes include catalase, all ascorbate-glutathione cycle components as well as superoxide dismutase (SOD). Interestingly, some of these antioxidant components are located in both the peroxisomal matrix and membrane.

The peroxisomal β-oxidation pathway, which generates significant amounts of $H_2O_2$, was first described in the germinating castor bean [18] and was later found in rat liver [66]. In germinating seeds, triglycerides are mobilized as a source of energy during the non-autotrophic stage prior to photosynthesis. Thus, fatty acids enter peroxisomes via ATP-binding cassette (ABC) transporters of subfamily D. They are then oxidized to fatty acetyl-CoA in peroxisomes and shortened by two carbons in each β-oxidation cycle. Finally, acetyl-CoA is converted to four-carbon molecules by the glyoxylate cycle, which then undergo gluconeogenesis in the mitochondrion and cytosol to provide energy for seedling development [14,5,78]. However, $H_2O_2$, which acts as a priming factor involving specific changes at the proteome, transcriptome and hormonal levels, is not simply a byproduct of peroxisomal β-oxidation [6,83,144].

Another important source of intracellular $H_2O_2$ is the peroxisomal glycolate oxidase (GOX), which is involved in photorespiration [48]. In addition to the importance of this pathway as a major determinant of $C_3$ crop biomass production, photorespiratory $H_2O_2$ appears to participate in pathogen defense [16].

Catalase (CAT) is one of the most representative peroxisomal antioxidant enzymes. While only one catalase isoform is encoded by a single gene in animal cells, catalase is present in the form of multiple isoforms encoded by a small gene family in plant cells [46,49,57]; consequently, the number and expression of various CAT isoforms change during plant development and under different environmental conditions. Given the oxidative metabolism of peroxisomes, various studies have evaluated the oxidative stability of plant catalase activity in the presence of high concentrations of $H_2O_2$ (up to 100 mM). However, its oxidation at multiple sites does, not affect catalase activity [2]. On the other hand, there is evidence to show that NO-donors, such as *S*-nitrosoglutathione (GSNO) and DETA NONOate, as well as nitrating agents, such as peroxynitrite ($ONOO^-$), cause the down-regulation of catalase activity ([15,17,87]). This inhibition of catalase activity could reflect a reduced capacity to remove $H_2O_2$ and consequently an increase in the nitro-oxidative metabolism [21,22]. This behavior has been described during the natural senescence of leaves and pepper fruit ripening, when both NO and catalase activity decline [15,24]. In this context, catalase can be regarded as a key enzyme involved in increasing cell longevity [36].

The antioxidant enzyme superoxide dismutase (SOD), which catalyzes the disproportionation of $O_2^{\cdot-}$ into $H_2O_2$ and $O_2$, is also present in peroxisomes. SOD, specifically Mn-SOD, was first described in plant peroxisomes in 1983 [43], whose presence was strongly questioned until CuZn-SOD was described in animal peroxisomes many years later [61]. Since then, an increasing number of reports have confirmed the presence of SOD in peroxisomes (see Table 2), which is now universally accepted as a key antioxidant enzyme in all types of peroxisomes. However, it is worth mentioning two important characteristics of the peroxisomal SOD. While CuZn-SOD has been exclusively found in the matrix of animal peroxisomes, the number, type and localization (matrix and/or membrane) of SOD in plant peroxisomes can change

**Table 2.**
Superoxide dismutases (SODs) localized in plant and animal peroxisomes.

| Source | SOD Isozyme | Reference |
|---|---|---|
| **Plants** | | |
| Pea | Mn-SOD | [43,90] |
| Watermelon | CuZn-SOD, Mn-SOD | [100,104] |
| Carnation | Fe-SOD, Mn-SOD | [39] |
| Castor bean | Mn-SOD | [41] |
| Sunflower | CuZn-SOD | [33] |
| Cucumber | CuZn-SOD, Mn-SOD | [33] |
| Cotton | CuZn-SOD | [33] |
| Tomato | SOD | [79] |
| Olive | CuZn-SOD | [28,120] |
| Pepper | Mn-SOD | [77] |
| | Fe-SOD | [91] |
| Rice | CuZn-SOD | [110] |
| **Animals** | | |
| Humans | | |
| Hepatoma cells | CuZn-SOD | [61] |
| Fibroblast | CuZn-SOD | [61] |
| Rat | | |
| Liver | CuZn-SOD | [37] |
| Brain | CuZn-SOD | [80] |
| Fish liver | CuZn-SOD | [86] |
| Molluscs digestive gland | CuZn-SOD | [86] |
| Crustaceans digestive gland | CuZn-SOD | [86] |

depending on the plant species (see Table 2). Moreover, to our knowledge, none of the SOD proteins localized in peroxisomes contain a classic PTS1 or PTS2. In this context, peroxisomal CuZn-SOD in mammals has been demonstrated to use a piggyback import mechanism, where physiological interaction with the copper chaperone of SOD (CCS) functions as a shuttle [51].

The ascorbate-glutathione (Asc-GSH) cycle constitutes a complementary system which enables plants to control $H_2O_2$ content. This cycle is composed of 4 enzymes: ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR), as well as antioxidants ascorbate and glutathione, and also requires NADPH as a reducing agent. Although the Asc-GSH cycle was initially found in cytosol and chloroplasts, these enzyme components have also been reported in plant peroxisomes, with APX being the first enzyme found to be present in peroxisomal membranes [13,26,117]. The other components were later identified and characterized in the peroxisomes of different plant species [59,77,81,102]. It is important to point out that the subcellular localization of the Asc-GSH cycle is peculiar to peroxisomes, as, while the APX enzyme is located on the outer surface of the membrane, the GR and DHAR enzymes are present in the matrix, and the MDAR enzyme is located in both the matrix and membrane [67,72]. Although the peroxisomal Asc-GSH cycle is involved in the mechanism of response to environmental stresses (for review, see [98]), it also appears to protect oil bodies close to peroxisomes from oxidative damage [45].

Nitric oxide (NO) is a free radical messenger belonging to a family of related molecules called reactive nitrogen species (RNS). These include molecules such as *S*-nitrosothiols (SNOs), *S*-nitrosoglutathione (GSNO), peroxynitrite ($ONOO^-$) and nitro-fatty acids ($NO_2$-FA), which directly or indirectly perform a broad spectrum of regulatory functions in many physiological and pathological processes in higher plants [31,38,76,121]. It is worth noting that plant peroxisomes were the first organelles found to show the presence of L-arginine-dependent nitric oxide synthase, a NOS-like activity with biochemical requirements (L-Arg, NADPH, FMN, FAD, CaM and $Ca^{2+}$) similar to those of animal NOS [7]. Interestingly, years later, NOS activity was also reported to be present in animal peroxisomes [74,109]. However, no ortholog genes analogous to classic mammalian NOSs have been found in higher plants, an issue which is still highly controversial in plant research. In this context, it has been crucial to demonstrate the presence of NO, the product of NOS-like activity. Various experimental approaches, such as spin-trapping electron paramagnetic resonance (EPR) spectroscopy and ozone chemiluminiscence assays, have enabled NO to be detected in isolated peroxisomes [24]. Later, it became possible to visualize peroxisomal NO production *in vivo* with the aid of specific, cell-permeable fluorescent probes to detect NO such as DAF-FM DA and DAR-4M AM. Confocal laser scanning microscopy (CLSM) was also used to analyze *Arabidopsis thaliana* transgenic plants expressing green or cyan fluorescent proteins by constructing peroxisomal targeting signal 1 (PTS1) [26]. Fig. 1 shows the visualization of NO and $ONOO^-$ in the guard cells of transgenic *Arabidopsis thaliana* expressing CFP-PTS1. Another set of experimental data which evidence the RNS metabolism in plant peroxisomes are related to the identification of peroxisomal proteins which undergo RNS-mediated post-translational modifications, including nitration and S-nitrosylation and affect peroxisomal protein function (Table 3).

Additionally, genetic techniques using Arabidopsis knock out mutants have shown that peroxisomal NO is generated by an NOS-like protein. By using Arabidopsis *pex12* and *pex13* mutants, it has been possible to demonstrate the absence of peroxisomal NO generation, suggesting that an NOS-like protein is involved [27]. Moreover, analysis of peroxisomal NO content in Arabidopsis *pex5* and *pex7* knockdown mutants subjected to RNA interference (RNAi) called *pex5i* and *pex7i*, respectively, enabled us to pinpoint the NOS-like protein responsible for NO generation in peroxisomes, which was imported via the PTS2 pathway [21,22]. Similar findings were also obtained with respect to the iNOS peroxisomal isoform present in rat hepatocytes, which is also imported through the PTS2 pathway [73], suggesting that both plants and animals contain a similar mechanism for importing the protein responsible for NO generation into peroxisomes.

## 3. Roles of peroxisomal ROS/RNS under physiological and stress conditions

The metabolic plasticity of plant peroxisomes is caused by their specific functions in various physiological processes and by their involvement in the mechanism of response to adverse environmental conditions.

### 3.1. Physiological conditions: seed and pollen germination, stomatal movement, senescence and fruit ripening

Peroxisomes are key elements in seed germination, as seedling growth in the dark requires the conversion of fatty acids to sugars through β-oxidation and gluconeogenesis [50,52,103]. During this process, large amounts of $H_2O_2$ are produced, thus promoting seed germination [114] which boosts the production of endogenous ethylene and leads to cell elongation in the root tip [56]. On the other hand, excess peroxisomal $H_2O_2$ appears to be regulated by the membrane-bound components of the ascorbate- glutathione (APX and MDAR) cycle in order to protect oil bodies against oxidative damage, which can inactivate the triacylglycerol lipase sugar-dependent1 and prevent the supply of carbon for seedling establishment [45]. Although seed germination is also stimulated by NO [9,64], the direct involvement of peroxisomal NO has not yet been confirmed. However, peroxisomal NO has been demonstrated to mediate auxin-induced lateral root organogenesis [105]. On the other hand, peroxisomal NO is directly involved in pollen germination and tube growth, and its target to the ovule [94–96].

In leaves, stomatal movement is highly regulated by external stimuli (light, $CO_2$ levels, water balance and pathogens) and also by internal molecules including hormones (ABA and salicylic acid) and ROS/RNS ($H_2O_2$ and NO) [10,82,111]. Fig. 1B shows the presence of

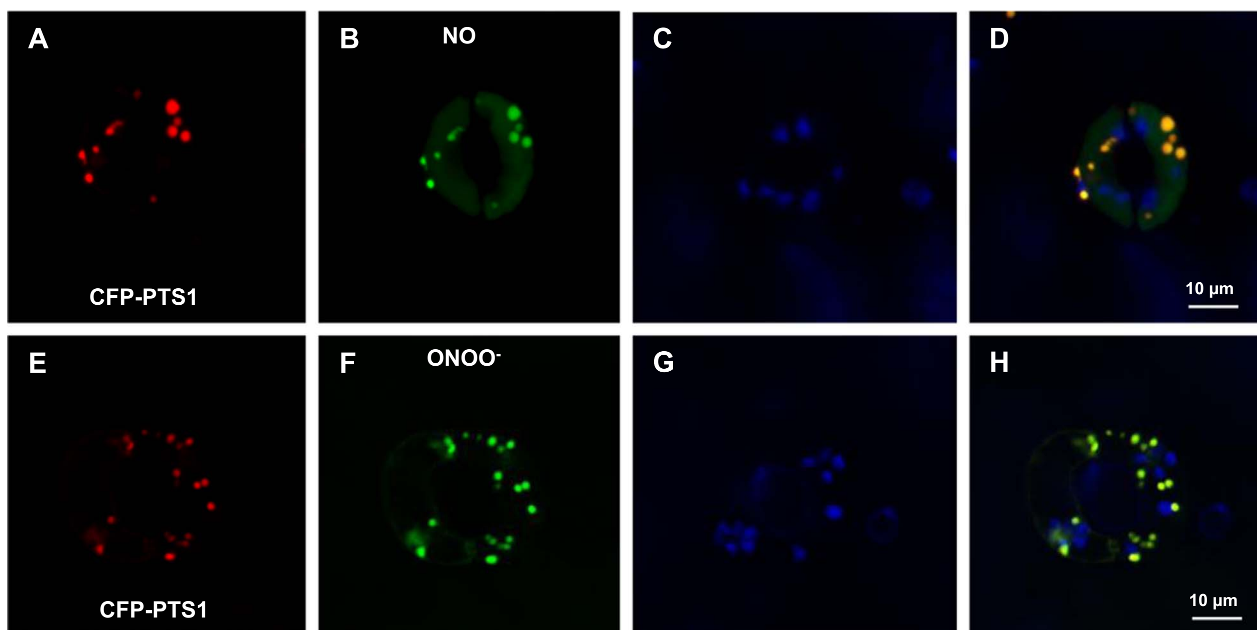


**Fig. 1.** Images illustrating CLSM *in vivo* detection of nitric oxide (NO) and peroxynitrite (green), peroxisomes (red) and chloroplasts (blue) in guard cells of transgenic Arabidopsis seedlings expressing CFP-PTS1. A and E, fluorescence punctates (red) attributable to CFP-PTS1, indicating the localization of peroxisomes in guard cells. B and F, fluorescence punctates (green) attributable to the detection in the same guard cells of nitric oxide and peroxynitrite, respectively. C and G, chlorophyll autofluorescence (blue) attributable to the detection of chloroplasts. D and H, merged images for corresponding panels.

**Table 3**
Summary of identified plant peroxisomal proteins susceptible of post-translational modifications mediated by RNS.

| NO-derived post-translational modification | Peroxisomal enzyme | Identification |
|---|---|---|
| Tyrosine nitration | Catalase<br>Monodehydroascorbate reductase[c]<br>Glycolate oxidase<br>Malate dehydrogenase,Hydroxypyruvate reductase[d] | Immunoreactive with antibody against nitrotyrosine[a] |
| S-nitrosylation | Catalase<br>Monodehydroascorbate reductase[c]<br>Glycolate oxidase<br>Malate dehydrogenase,<br>Hydroxypyruvate reductase | Preincubation with GSNO (NO donor) and biotin-switch[b] |

[a] Lozano-Juste et al., 2011 [75]; Chaki et al., 2015 [15].
[b] Ortega-Galisteo et al., 2012 [87].
[c] Begara-Morales et al., 2015 [8].
[d] Corpas et al., 2013 [30]

NO (green color) in peroxisomes and the cytosol. Although it has been well established that NO induces stomatal closure as part of a mechanism of response to water deficit [82] and also restricts the entry of pathogenic microorganisms [1], the direct involvement of peroxisomal NO has not yet been confirmed. In this respect, it has been reported that Arabidopsis peroxisomal NADP-isocitrate dehydrogenase (ICDH) knock out mutants show a reduced stomatal aperture as compared to wild type plants [68]. The fact that NADPH generated by this enzyme is necessary for peroxisomal NO generation can be used as indirect evidence of the involvement of peroxisomal NO in stomatal movement. ROS have also been shown to be involved in stomatal movement. Thus, in maize plants grown under drought stress conditions, high accumulation rates of $H_2O_2$ in guard cells have been reported to result in stomatal closure [116]. This closely correlates with the regulation of Arabidopsis catalase 3, which, in turn, enables $H_2O_2$ to be modulated in stomatal guard cells [123].

Peroxisomal NO and ROS are also involved in leaf senescence, which is characterized by programmed degeneration controlled by multiple developmental and environmental signals. This process is mainly characterized by a decline in photosynthesis, marked chlorophyll and protein loss, disintegration of organelle structures and a dramatic increase in lipid peroxidation where the imbalance between ROS production and antioxidative systems in the different subcellular compartments is significantly affected [12,89,92]. As the senescence of Arabidopsis leaves progresses, the isoforms Cat2 and Cat3 have been shown to decrease, which could be a signal for cells to promote senescence [122]. Similarly, during the natural senescence of pea leaves, the decrease in catalase activity is accompanied by a down-regulation of NO generation [24]. A similar observation has been made during pepper fruit ripening, which can be regarded as a process of senescence, where catalase activity is also down-regulated and is accompanied by increased nitration [15]. Furthermore, Arabidopsis mutants called *peup* (peroxisome unusual positioning), characterized as containing aggregated peroxisomes, have also been found to contain high levels of oxidized proteins and inactive catalase. These aggregates are damaged peroxisomes which are selectively degraded by pexophagy at the senescence stage [107]. This suggests that $H_2O_2$ is a signal for pexophagy which contributes to the removal of damaged peroxisomes in the cell [118]. However, other data indicate that misfolded or aggregated peroxisomal matrix proteins may be cellular signals for pexophagy, where the protease LON2 is involved in protein quality control [119].

### 3.2. Stress conditions: salinity and heavy metals (Cd and lead)

The involvement of the plant peroxisomal ROS metabolism in the mechanism of response to biotic and abiotic stress has been confirmed by numerous examples (see [20]). However, less information is available on the involvement of peroxisomal NO. In this context, several studies have provided sufficient evidence to indicate that, under certain environmental stress conditions such as salinity and heavy metals, the peroxisomal NO metabolism is significantly triggered, resulting in the generation of cellular nitro-oxidative stress (Table 4).

In *Arabidopsis thaliana* seedlings grown under salinity stress

**Table 4**
Summary of the main functions where the peroxisomal nitric oxide is involved.

| Plant process | Reference |
|---|---|
| Pollen tube growth | [94] |
| Senescence | [24] |
| Auxin-induced root organogenesis | [105] |
| Abiotic stress | |
| Salinity [100 mM NaCl] | [29] |
| Cadmium [150 µM $CdCl_2$] | [22] |
| Lead [150 µM $Pb(NO_3)_2$] | [23] |

(100 mM NaCl), an increase in peroxisomal NO content has been reported. This also promotes its accumulation in the cytosol, thus contributing to the generation of $ONOO^-$ and consequently to an increase in protein tyrosine nitration [29], which is regarded as a marker of nitrosative stress [27]. Similarly, in Arabidopsis seedlings exposed to cadmium (150 µM) stress, peroxisomal NO content was found to increase, which was accompanied by increased superoxide radical content and, accordingly, higher peroxynitrite production [21,22]. Likewise, Arabidopsis grown in the presence of 150 µM lead ($Pb^{2+}$) also caused NO, $O_2^{\cdot-}$ and $ONOO^-$ overproduction. Furthermore, biochemical and gene expression analyses of peroxisomal enzymes, including the antioxidant catalase and two photorespiration enzymes glycolate oxidase (GOX) and hydroxypyruvate reductase (HPR), have shown that only the catalase was clearly affected under $Pb^{2+}$ stress, while both photorespiratory enzymes were unaffected. These findings corroborate the involvement of plant peroxisomal metabolisms in the mechanism of response to lead contamination and highlight the importance of the peroxisomal NO metabolism [23] under abiotic stress conditions.

## 4. Conclusions

In plants, RNS and ROS are two families of related molecules generated in the cellular metabolism where peroxisomes are important organelles. Molecules, such as NO, GSNO and $H_2O_2$, which are endogenously produced by peroxisomes, can act as signal elements involved in different transduction pathways of cellular communication. Fig. 2 summarizes the principal elements involved in the endogenous peroxisomal metabolism of NO/$H_2O_2$, which can diffuse out of these organelles and affect other cell compartments. Nitric oxide generated from L-arginine by NOS-like activity interacts with superoxide radicals ($O_2^{\cdot-}$) to generate peroxynitrite ($ONOO^-$), which can mediate the protein tyrosine nitration of specific peroxisomal target proteins (Table 3). On the other hand, NO can react with reduced glutathione (GSH) to produce *S*-nitrosoglutathione (GSNO) which, through a process of transnitrosylation, affects other peroxisomal enzymes (Table 3). Although, little is known about the permeability of GSNO through cellular membranes [11,101], it is possible to suggest that NO, and perhaps GSNO, is released through the peroxisomal membrane to the cytosol and initiates a signaling cascade or interacts with other biomolecules; this causes post-translational modifications or is part of a mechanism of response to various types of stresses. Hydrogen peroxide is produced via different biochemical pathways, such as β-oxidation and photorespiration, in plant peroxisomes. Additionally, $O_2^{\cdot-}$ generated by certain enzymes, such as xanthine oxidase (XOD, part of the purine metabolism) [32], can be dismutated to $H_2O_2$ by the

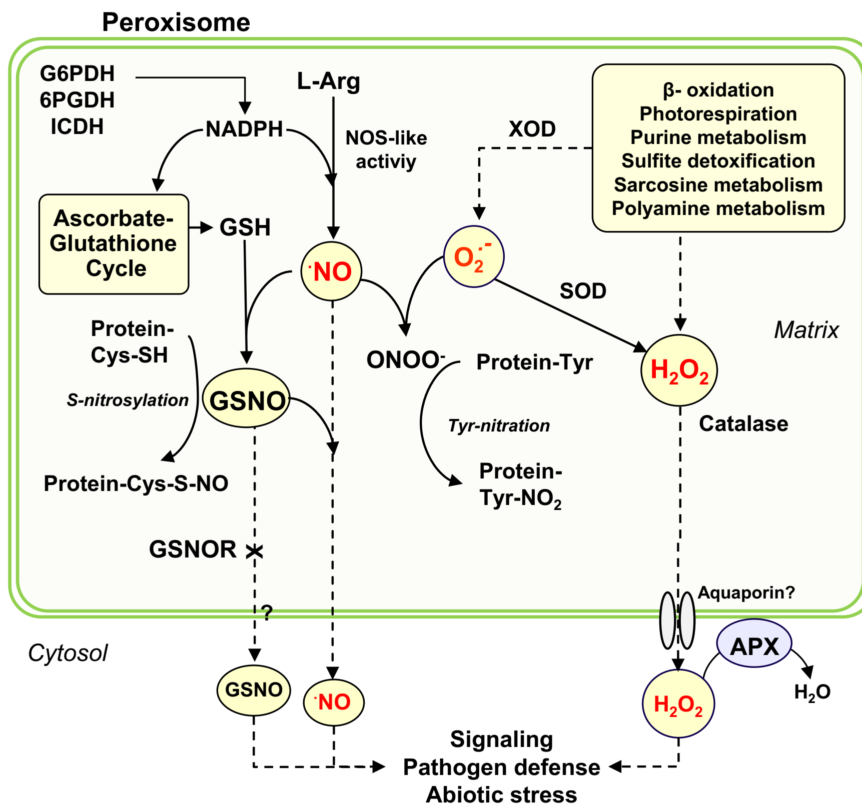


**Fig. 2.** Model of NO and $H_2O_2$ metabolism in plant peroxisomes. L-Arginine nitric oxide synthase (NOS)-like activity generates NO which can react with reduced glutathione (GSH) in the presence of $O_2$ to form *S*-nitrosoglutathione (GSNO). This metabolite can interact with SH-containing proteins by a process of S-nitrosylation affecting their function. NO can also react with superoxide radicals ($O_2^{\cdot-}$) to generate peroxynitrate ($ONOO^-$) which can mediate a process of tyrosine nitration of proteins. Alternatively, either NO or GSNO can be released to the cytosol to participate in signaling cascades, although the *S*-nitrosoglutathione reductase (GSNOR) could modulate the GSNO release. Hydrogen peroxide ($H_2O_2$) is produced by different biochemical pathways. Additionally, the superoxide radical ($O_2^{\cdot-}$) generated by some enzymes such as xanthine oxidase (XOD) which is part of purine metabolism can dismutate into $H_2O_2$ by the enzyme superoxide dismutase (SOD). The level of peroxisomal $H_2O_2$ is controlled either by catalase located in the matrix or by the membrane bound ascorbate peroxidase (APX), and its release to the cytosol could be through putative aquaporins.

enzyme superoxide dismutase (SOD). Although, peroxisomal $H_2O_2$ levels are controlled by catalase located in the matrix or by membrane-bound ascorbate peroxidase (APX), this does not discard the potential presence of $H_2O_2$-transporting aquaporins in the peroxisomal membrane [115]. Additionally, in this peroxisomal model, it is also important to mention the presence of other ascorbate-glutathione cycle components (MDAR, DHAR and GR) as well as a group of NADPH regenerating enzymes glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH) and NADP-depending isocitrate dehydrogenase (NADP-ICDH). This group of enzymes provides the NADPH necessary for both GR and L-arginine NOS-like activities.

In summary, peroxisomal studies have opened up exciting new avenues of investigation for the community of researchers working on cellular integration. Molecules such as NO and $H_2O_2$ act as retrograde signals among the different cellular compartments and also regulate gene expression which facilitates cellular adaptation to environmental changes. We also believe that the data obtained from plant peroxisomes will be useful for understanding the important role played by these organelles in the eukaryotic organisms of animals and yeast among others.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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