Nitro-fatty acids in plant signaling: New key mediators of nitric oxide metabolism

Capilla Mata-Pérez, Beatriz Sánchez-Calvo, Maria N. Padilla, Juan C. Begara-Morales, Raquel Valderrama, Francisco J. Corpas, Juan B. Barroso

Abstract

Recent studies in animal systems have shown that NO can interact with fatty acids to generate nitro-fatty acids (NO2-FAs). They are the product of the reaction between reactive nitrogen species and unsaturated fatty acids, and are considered novel mediators of cell signaling based mainly on a proven anti-inflammatory response. Although these signaling mediators have been described widely in animal systems, NO2-FAs have scarcely been studied in plants. Preliminary data have revealed the endogenous presence of free and protein-adducted NO2-FAs in extra-virgin olive oil (EVOO), which appear to be contributing to the cardiovascular benefits associated with the Mediterranean diet. Importantly, new findings have displayed the endogenous occurrence of nitrolinolenic acid (NO2-Ln) in the model plant Arabidopsis thaliana and the modulation of NO2-Ln levels throughout this plant’s development. Furthermore, a transcriptomic analysis by RNA-seq technology established a clear signaling role for this molecule, demonstrating that NO2-Ln was involved in plant-defense response against different abiotic-stress conditions, mainly by inducing the chaperone network and supporting a conserved mechanism of action in both animal and plant defense processes. Thus, NO2-Ln levels significantly rose under several abiotic-stress conditions, highlighting the strong signaling role of these molecules in the plant-protection mechanism. Finally, the potential of NO2-Ln as a NO donor has recently been described both in vitro and in vivo. Jointly, this ability gives NO2-Ln the potential to act as a signaling molecule by the direct release of NO, due to its capacity to induce different changes mediated by NO or NO-related molecules such as nitration and S-nitrosylation, or by the electrophilic capacity of these molecules through a nitroalkylation mechanism. Here, we describe the current state of the art regarding the advances performed in the field of NO2-FAs in plants and their implication in plant physiology.

1. Introduction

Electrophioph nitro-fatty acids (NO2-FAs) are endogenously formed by redox reactions of nitric oxide (NO) and NO-derived molecules that promote the generation of nitro dioxide (NO2), which in turn nitrates unsaturated fatty acids [1–3]. The first report describing the endogenous occurrence of lipid nitrated derivatives was defined both in normolipidemic and hyperlipidemic donors [4]. Numerous studies have since reported a selective nitration of fatty acids under physiological conditions, highlighting the greater susceptibility to nitration of conjugated fatty acids than methyl-interrupted dienes or polyenes in animal systems [5]. Most of these analyses have shown that these molecules trigger pleiotropic signaling actions including a remarkable anti-inflammatory and antioxidant response [6–9]. A measurable increase in the levels of NO2-FAs has been shown under different pathological conditions such as ischemia reperfusion injury or after LPS injection in the peritoneum [1,10].

Currently, the formation mechanisms of NO2-FAs remain unknown although different ways have been proposed, such as by NO2-, peroxynitrite (ONOO-) or protonation of nitrite (NO2-) yielding nitrous acid (HNO2) and subsequent nitrating and nitrosating species [11]. By contrast, several ways of inactivating nitro-fatty acid signaling have recently been described. In this regard, the NADPH-dependent enzyme prostaglandin reductase-1 (PGR-1) is involved in the inactivation of...
NO₂-FAs in the liver through a saturation mechanism [12]. Moreover, conjugation of these molecules to reduced glutathione (GSH) and subsequent β-oxidation and excretion through urine has also been noted as a mechanism of inactivation of NO₂-FAs [13]. Furthermore, due to the hydrophobic nature of these molecules, cell membranes and/or lipoproteins may constitute a natural source of NO₂-FAs [14,15]. Based on the electrophilic capacity of nitro-fatty acids, an important part of the endogenous content of these molecules could also be found adducted with different nucleophiles, mainly with proteins [16]. In fact, the intravenous injection of nitro-oleic acid (NO₂-OA) in mice has shown that this NO₂-FA is rapidly adducted to plasma thiol-containing proteins and glutathione, and this being only a small portion of free NO₂-OA in plasma [1]. Furthermore, it has been recently described the release of NO₂-FAs from Cys-adducted nitroalkenes under nitro-oxidative stress situations [17]. Representative ROS and RNS, such as hydrogen peroxide (H₂O₂) and ONOO⁻ respectively, are able to initiate the release of free nitroalkenones from protein-adducted NO₂-FAs. These nitro-oxidative conditions promote the formation of a β-nitrosulfoxide intermediate and consequently releasing free NO₂-FAs. This behavior may have relevance under several stressful situations both in animal and plant systems such as inflammation or under different adverse environmental conditions in which an increase of ROS and RNS has been noted [18–22]. Actually, the anti-inflammatory and antioxidant signaling properties of these molecules have been attributed to free NO₂-FAs [23–27].

The beneficial properties of NO₂-FAs described above have been attributed mainly to the electrophilic capacity of the β-carbon of the reactive nitroalkenyl substituent from NO₂-FAs, which readily undergoes a reversible Michael addition [2,28,29]. This reaction is known specifically as nitroalkylation and occurs primarily with functionally significant Cys and His residues on transcription-regulating proteins and enzymes (Fig. 1) [2,16,28]. Through this post-translational alteration, NO₂-FAs can reportedly exert pleiotropic signaling actions, particularly an anti-inflammatory and antioxidant response [2,29]. In this respect, electrophilic nitroalkenones such as nitro-oleic (NO₂-OA) or nitro-linoleic (NO₂-Ln) acids have been identified as antagonists of the nuclear lipid receptor peroxisome proliferator-activated receptor-γ (PPARγ) [30,31], the Keap1-Nrf2 signaling pathway [6,32], and the heat-shock response [26]. Furthermore, the antagonistic properties of NO₂-FAs on NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) also promote a strong anti-inflammatory response [23].

On the other hand, the ability of NO₂-FAs to release NO has been thoroughly described [33–35], but the precise mechanism of NO release has not yet been identified. Nevertheless, [34] postulated that NO release from NO₂-Ln may be based on a modified Nef reaction [34]. In this sense, deprotonation of the carbon γ to the nitro group leads to the formation of an unstable nitroso intermediate that rapidly degrades to release either HNO, the predicted Nef reaction product, or NO, a product considered likely due to the weak C-N bond present on NO₂-FAs [2,28,29]. This group could also promote the formation of nitrous acid (HNO₂) in the stomach being thereby broken down to NO₂. Interestingly, the release of NO from NO₂-FAs may contribute to the cardiovascular benefits associated with the Mediterranean diet [27].

2. Characterization of the endogenous occurrence of NO₂-FAs in plant systems

2.1. Extra-virgin olive oil (EVOO) and olives

The Mediterranean diet constitutes a key source of unsaturated fatty acids with extra-virgin olive oil (EVOO) providing health benefits to humans. The Mediterranean diet is associated with different anti-inflammatory and anti-hypertensive effects leading to a reduced risk of cardiovascular morbidity and mortality [40–42]. Based on the previously reported anti-inflammatory properties attributed to NO₂-FAs, an initial study analyzing the endogenous occurrence of these molecules was made in EVOO and fresh olives [43]. In this respect, the authors noted the presence of nitrated derivatives of conjugated linoleic acid (NO₂-cLA) in EVOO, specifically of 9- and 12-NO₂-c-LA isomers. Moreover, the Mediterranean diet is also characterized by the high intake of leafy vegetables and cured meats, which are rich in nitrates (NO₃⁻) and nitrates (NO₂⁻). NO₃ can be reduced by bacteria in the saliva to NO₂⁻ and the latter can form nitrous acid (HNO₂) in the stomach being thereby broken down to NO₂. Furthermore, oils, fish, several seeds, and dairy products such as milk or cheese are rich sources of ω-3 and ω-6 fatty acids, including oleic, linoleic and linolenic acids. These factors together with acidic conditions of digestion may lead to the gastric generation of NO₂-FAs. In this regard, the incubation of EVOO with gastric juice artificial and ¹⁵N-labelled sodium nitrite (Na[¹⁵N]NO₂) results in the detection of different NO₂-FAs such as 9- and 10-NO₂-OA; 9/10-, 12- and 13-NO₂-LA and 8-, 9-, 11- and 12-NO₂-c-LA [43]. Finally, NO₂-OA-cysteine adducts have been endogenously detected in acid-hydrolyzed proteins from the peel and mesocarp of freshly picked whole olives of different cultivars. For example, NO₂-OA-cysteine levels were higher in Picial olive cultivars and more abundant in the peel from these olives [43], underscoring that EVOO and olives are both a source and metabolic reserve of NO₂-FAs.

Importantly, the ex vivo detection of NO₂-OA and NO₂-LA in EVOO may be responsible in part for the numerous beneficial properties attributed to this important source of fatty acids in the Mediterranean diet. Furthermore, nitrated derivatives of oleic and linoleic acids not only inhibit leukocyte and platelet activation [44], vascular smooth-muscle proliferation [6], and lipopolysaccharide-stimulated macrophage cytokine secretion [23], but also activate PPARy receptor [45] and induce endothelial heme oxygenase 1 expression [46]. These NO₂-FAs also strongly modulate Nrf2/Keap1 [6,23] and NF-κB-regulated inflammatory signaling [23], as previously mentioned. Therefore, the presence of nitrated derivatives of oleic, linoleic or conjugated linoleic acids may contribute to the cardiovascular benefits associated with the Mediterranean diet.

2.2. The model plant Arabidopsis thaliana

Previous findings on the endogenous occurrence of NO₂-FAs in EVOO and olives prompted the analysis of the presence of these molecules in the model plant arabinidopsis (Arabidopsis thaliana). The study was performed using mass spectrometric approaches in 9-ω-old arabidopsis cell-suspension cultures (ACSC) and in 14-ω-old seedlings [27]. Firstly, lipid composition analysis of 9-ω-old ACSC and 14-ω-old seedlings revealed that linolenic acid (18Δ3) was the most abundant unsaturated fatty acid in both samples followed by linoleic (18Δ2) and oleic (18Δ1) acids. This background motivated the study of the presence of nitrated derivatives of the most abundant unsaturated fatty acids, and therefore an analysis was made of the presence of NO₂-OA, NO₂-LA and nitro-linolenic acid (NO₂-Ln). Synthesis of these different standards by a nitroselenation procedure made it possible to

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identify the only endogenous occurrence of NO2-Ln by comparing the retention time of the chromatographic peak of NO2-Ln standard to those observed in both 9-D-old ACSC and 14-D-old seedlings [27]. As confirmation that the peak detected corresponded to electrophilic NO2-Ln, a nitroalkylation reaction with β-mercaptoethanol (β-ME) of the lipid extract was carried out by seeking the covalent nitroalkylated adduct formed by the reaction of NO2-Ln and the thiol of β-ME. After incubation, the retention time of the peaks detected changed, confirming the electrophilic capacity of NO2-Ln and showing the endogenous presence of this nitro-fatty acid in arabidopsis. Furthermore, the NO2-Ln content was analyzed in both samples (Table 1) and the results showing 0.28 ± 0.04 pmol/g FW in 9-old ACSC and 3.84 ± 0.44 pmol/ g FW in 14-D-old seedlings [27]. These low levels were consistent with previous results found in the field of animal systems for other nitro-fatty acids such as NO2-OA [47] and NO2-LA suggesting these molecules could act as important signaling components of plant physiology. Moreover, the presence of other nitrated derivatives from oleic, linoleic and conjugated-linoleic acids was also analyzed. In all studied samples, the endogenous occurrence of nitro-oleic acid (MRM transition 326/279 m/z) was not observed due to no chromatographic peaks were detected with this MRM transition. Regarding the presence of nitro-linoleic or nitro-conjugated linoleic acids with MRM transition 324/277 m/z, a chromatographic peak with this MRM transition was observed. Nevertheless, the incubation of lipid extracts from all the analyzed samples with the electrophile β-ME did not make disappear those chromatographic peaks assuring they did not correspond to any of these NO2-FAs.

### 2.3. Nitro-fatty acid detection in other plant species

Besides showing the endogenous presence of NO2-Ln in the arabidopsis, the detection of NO2-FAs in other plant species was also analyzed (Table 1). In line with the approaches followed in arabidopsis, nitrated derivatives from the most abundant unsaturated fatty acids were found. This analysis resulted in the endogenous detection of NO2-Ln in roots from pea (Pisum sativum) plants and in leaves from rice (Oryza sativa) plants, the levels of this nitro-fatty acid being 0.072 and 0.748 pmol/ g FW, respectively (Table 1). Mitochondrial and peroxisomal fractions from pea plants were also subjected to the analysis of the different NO2-FAs, revealing NO2-Ln in both samples. The content of this molecule was about 0.084 and 0.282 pmol/g FW, in the mitochondrial and peroxisomal fractions, respectively (Table 1). In all analyzed samples was only possible to detect the endogenous

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**Table 1**

Endogenous detection of nitro-linolenic acid (NO2-Ln) in several plant species and in different subcellular fractions. ACSC, Arabidopsis cell suspension cultures.

| Plant species          | Organ/Subcellular fraction | pmol NO2-Ln/ g FW |
|------------------------|----------------------------|-------------------|
| Arabidopsis thaliana   | 14-old seedlings           | 3.84              |
|                        | 9-old ACSC                 | 0.28              |
| Pea (Pisum sativum)    | Roots                      | 0.072             |
|                        | Leaves                     | 0.084             |
|                        | Mitochondria               | 0.282             |
|                        | Peroxisomes                |                   |
| Rice (Oryza sativa)    | Leaf                       | 0.748             |
occurrence of NO$_2$-Ln, but not other nitrated derivatives from major fatty acids such as NO$_2$-OA or NO$_2$-LA, suggesting the higher susceptibility of linolenic acid to be endogenously nitrated and the relevance of this NO$_2$-FA in plant systems. All these results also highlight a ubiquitous distribution of NO$_2$-FAs in plant kingdoms and the potential signaling actions of these molecules in plant systems.

3. Involvement of nitro-fatty acids at development and under abiotic stress

In light of the outcomes of the *A. thaliana* analyses, the study concerning the levels of NO$_2$-Ln was extended throughout plant development and under different stress conditions, specifically abiotic stress. Furthermore, based on the low levels of NO$_2$-Ln in plant systems, a transcriptomic analysis with RNA-seq technology helped to elucidate the potential signaling role of this molecule in plant physiology.

3.1. Modulation of NO$_2$-Ln content throughout the development of Arabidopsis

As mentioned above, the endogenous occurrence of NO$_2$-Ln was observed in 9-d-old ACSC and in 14-d-old seedlings. These results prompted an analysis of the content of NO$_2$-Ln throughout the developmental process of arabidopsis. In this sense, different arabidopsis plant materials such as seeds, 14-d-old seedlings, and leaves from 30- and 45-d-old plants, with clear symptoms of senescence the latter, were used for this study. Higher levels of NO$_2$-Ln were found at the beginning of the development, specifically in seeds (11.18 ± 1.68 pmol/g FW) and in 14-d-old seedlings (3.84 ± 0.44 pmol/g FW). Nevertheless, this content significantly declined with the progress of plant development and with senescence in leaves from 30- (0.36 ± 0.04 pmol/g FW) and 45-d-old plants (0.54 ± 0.06 pmol/g FW) [27]. Nitro-fatty acids have been demonstrated to be in vitro and in vivo NO donors [33–35,46]. NO has a major role at the beginning of plant development because this molecule triggers seed germination [49], among other key processes in plant physiology. The higher content of NO$_2$-Ln detected in seeds may imply that this nitro-fatty acid acts as a NO donor at this stage and therefore favors germination and the onset of vegetative development. In this respect, it has been recently shown that S-nitrosylation of the ABA-related transcription factor, ABI5 promotes the interaction with CUL4-based and KEG E3 ligases, which are subsequently degraded by the proteasome, promoting seed germination [50]. Therefore, it cannot be ruled out that NO$_2$-Ln could release NO in seeds and mediate a S-nitrosylation mechanism of key transcription factors such as ABI5, thereby stimulating seed germination.

3.2. Raising of NO$_2$-Ln levels under abiotic-stress situations

Besides the involvement of NO and other NO-related molecules in several physiological processes such as germination, growth, development, and senescence [22,49,51–56], this gaseous molecule is also involved in the response to several (a)biotic stress conditions such as pathogen infection, symbiotic interactions, mechanical wounding, salinity, UV-radiation, and heavy-metal stress [21,57–67]. In relation to this implication, the levels of NO$_2$-Ln were analyzed under different abiotic-stress conditions. With 9-d-old ACSC exposure to 100-nM NaCl, a significant rise of NO$_2$-Ln content was observed after 5 min of treatment (0.96 ± 0.12 pmol/g FW) compared to steady-state levels (0.28 ± 0.04 pmol/g FW), which tripled the levels of this nitro-fatty acid. Nevertheless, after 30 min of salt stress the levels of NO$_2$-Ln fell [27]. Moreover, in 14-d-old seedlings subjected to mechanical wounding, heavy-metal and low-temperature stress, a noteworthy rise in NO$_2$-Ln levels was recorded, values being 7.46 ± 1.20, 6.62 ± 0.98 and 5.75 ± 0.79 pmol/g FW, respectively, with regard to control seedlings (3.84 ± 0.44 pmol/g FW) [27]. These results indicate the involvement of NO$_2$-Ln in the response to different abiotic-stress situations.

4. Signaling role of NO$_2$-Ln in plant physiology

Because the NO$_2$-Ln content was modulated throughout plant’s development and under different stressful situations together with the low observed levels of this molecule in plants systems, a transcriptomic analysis was performed to define the potential signaling actions of this molecule in plant physiology. Furthermore, based on the implications of NO$_2$-Ln in a wide range of actions, the ability to release NO both *in vitro* and *in vivo* was also assessed.

4.1. Transcriptomic analysis of NO$_2$-Ln-responsive genes

To establish the potential signaling mechanisms in which NO$_2$-Ln was involved, a transcriptomic analysis by RNA-seq technology was performed by analyzing 10 µM and 100 µM concentrations of this nitro-fatty acid in 9-d-old ACSC [27]. The use of these concentrations of NO$_2$-Ln indicated a clear signaling response of this molecule in plant physiology, in agreement with concentrations used in previous studies in animal systems [13,47]. Importantly, the application of NO$_2$-Ln to ACSC prompted a dose-dependence response ranging from 1 µM to 100 µM NO$_2$-Ln which was shown by qRT-PCR and by DNAstar software [27], this having been previously described for NO$_2$-OA or NO$_2$-LA in animal systems [5,26].

Firstly, with the use of the Blast2GO suite, a Gene Ontology term-enrichment analysis of NO$_2$-Ln-responsive genes showed that over-expressed genes were related to stress response, but specifically with the response to abiotic stress and to oxidative-stress-related processes. By contrast, down-regulated genes were involved in some biological processes and especially with the biosynthesis of several cell compounds [27]. Therefore, these results suggest a close relationship between NO$_2$-Ln and the involvement in different abiotic-stress conditions. A subset of genes modulated by NO$_2$-Ln in ACSC were selected for a transcriptional analysis in other stages of plant development, further confirming the signaling capacity of this molecule in plant physiology. Results from this analysis show that NO$_2$-Ln application to 14- and 45-d-old arabidopsis plants followed the same trend both in *up*- and *down*-regulated genes, thus corroborating the signaling capacity of NO$_2$-Ln throughout the overall development of arabidopsis [27].

An in-depth analysis of genes modulated by NO$_2$-Ln indicated that up-regulated genes were involved mostly in protein folding and the response to heat and H$_2$O$_2$ [27]. In line with these results, a network graph analysis with GeneMANIA resource showed that around a 40% of the total genes induced by NO$_2$-Ln treatment corresponded to members of the heat-shock response (HSR). Among these were numerous small heat-shock proteins (sHSP), but also some heat-shock transcription factors (HSFA2 and HSFA7B) as well as members of HSP40, HSP60, HSP70, and HSP90 families. The only transcriptomic analysis made to date with NO$_2$-FAs in animal systems has been a microarray study with NO$_2$-OA in human endothelial cell cultures [26] showing a noteworthy activation of the HSR in an *Nrf2*-independent manner. This activation of the HSF pathway contributes to cell protection through signaling actions involved in mainly anti-inflammatory processes. Outcomes derived from the study of the involvement of NO$_2$-Ln in plant physiology resulted in a similar response to that previously observed with NO$_2$-OA in animal systems, therefore suggesting a conserved mechanism of action of NO$_2$-FAs both in animal and in plant systems. At this point, it is also important to bear in mind that incubation of ACSC with other NO$_2$-FAs such as NO$_2$-OA and NO$_2$-LA generated a similar transcriptional response to that of the same NO$_2$-FA in plant systems. All these results also highlight a possible role of nitro fatty acids in several physiological processes such as germination, growth, development, and senescence [22,49,51–56].
heavy metals, low temperature and mechanical wounding [27]. A general phenomenon underlying abiotic-stress situations is oxidative stress [68,69]. Based on GO-term analysis, NO2-Ln has been associated with the response to H2O2, to reactive oxygen species (ROS), and to oxygen-containing compounds [27]. Among genes related to these processes, the high induction of cytosolic ascorbate peroxidase 2 (APX2) was noted together with other members involved in different redox processes [27]. APX is a key component of plant-defense responses against oxidative stress because this enzyme is able to detoxify the H2O2 generated under different stressful situations using ascorbate as an electron donor. Furthermore, the close interaction between the HSF2A2 transcription factor and APX2 enzyme under stressful situations such as high temperature and light-intensity stress has been reported [70]. This nitro-fatty acid is able to act as a signaling mediator in the plant-defense mechanism in abiotic-stress situations, setting up a defense response against cell damage arising as a result of stress and mediated mainly by HSP induction. Therefore, these results suggest a close relationship with high temperature, abiotic stress in general, and oxidative stress—with both stress processes tightly regulated by NO2-Ln.

Finally, a high percentage of down-regulated genes were involved in the metabolism and decrease in sugar biosynthesis, in cell-wall components, and in chlorophyll, together with a decline in genes associated with photosynthesis, the electron-transport chain, and metabolite transport [27]. This phenomenon leads to a metabolic reconfiguration with a down-regulation in the biosynthesis of new components, counteracting energy costs and indicating is required to maintain a balance between the continuation of cell function and survival [71,72].

4.2. NO2-Ln is a NO donor

All the results previously described show a significant involvement of NO2-Ln in plant physiology and specifically in the defense response to adverse abiotic conditions. However, the mechanisms by which this nitro-fatty acid is able to launch this plant-defense response remain unknown. As has been described for NO2-OA or NO2-LA in animal systems, the ability of NO2-Ln to modulate the generation of NO has also been assessed in the Arabidopsis in order to define how NO2-Ln sets up this antioxidant mechanism. Firstly, several in vitro approaches were carried out to assess the capacity of NO2-Ln to generate NO. An initial study was performed using a spectrofluorometric assay with DAF-2 fluorochrome in phosphate buffer and in the presence of 5 µM of NO2-Ln. Outcomes derived from this analysis showed that this molecule was able to boost fluorescence due to NO release, this being reduced with the application of this molecule was able to boost fluorescence, thus confirming that NO2-Ln is a NO donor (pathway 2). Based on the key role of NO in a wide range of physiological and stress-response processes, the ability of NO2-Ln to act as a NO donor seems to be important in organelles and cell compartments where the pH is neutral or basic. All these results underscore the capability of NO2-Ln to be a NO donor and the possible implications of this molecule in a wide range of signaling actions in plant physiology.

Fig. 1 shows a schematic model of NO2-Ln signaling. This nitro-fatty acid can establish a defense mechanism and an antioxidant response against adverse environmental conditions through the induction in the expression of a large set of heat-shock proteins (HSPs) and several antioxidant systems such as ascorbate peroxidase (APX), among others (pathway 1). Furthermore, it has recently been shown that NO2-Ln is a NO donor (pathway 2). Based on the key role of NO in a wide range of physiological and stress-response processes, the ability of NO2-Ln to act as a NO donor may have great relevance in plants. In fact, given that the content of NO2-Ln suffers a significant increase under several abiotic stress situations such as mechanical wounding, salinity, low temperature or cadmium stresses and the initial stage of Arabidopsis development [27], the NO release from increased NO2-Ln could be mediating diverse signaling actions that supporting these processes. Therefore, the ability of NO2-Ln to act as a NO donor may be able to support the NO implication in plant development, different biotic or abiotic processes, the antioxidant response or even mediating several post-translational modifications (NO-PTMs) as nitration and S-nitrosylation through which NO releases its bioactivity [76–78]. In the latter case, NO released from NO2-Ln could bind to specific cysteine thiol groups of proteins thus promoting the formation of S-nitrosothiols (SNOs) and therefore be they implied in processes such as plant immunity [79,80] or the response to abiotic stress conditions [21,64,81]. In fact, most of the S-nitrosylated proteins involved in the response to abiotic stress situations are redox-related proteins, such as ROS generating enzymes or antioxidant systems, suggesting a role of S-
that, based on the ability of NO2-Ln to be a NO donor, this molecule activity, thus preventing the oxidative stress generated under this nitrosylation process of APX enzyme and hence increasing the enzyme of this molecule to release NO has also been reported, the precise modi
c anti-in

Results obtained in animal systems have shown that nitroalkylation of oxidant peroxynitrite (ONOO−) [82]. This RNS could be mediating conditions, because free NO2-FAs may be able to activate the relevant the content of ROS and RNS take place, the oxidation of the Michael adduct and subsequent release of free nitroalkenes could occur (pathway 4). This could have important signaling implications under stress conditions, because free NO2-FAs may be able to activate the relevant antioxidant properties that these molecules possess. Finally, the observed increase in the expression of APX could help alleviate the oxidative stress generated under stressful conditions by detoxifying the H2O2 produced under these circumstances.

5. Conclusions and future perspectives

A significant advance in understanding the interaction between NO and NO-derived molecules with unsaturated fatty acids has been achieved in animal systems. This interaction has started to be studied very recently in plant systems with the endogenous occurrence of nitrated derivatives of linoleic acid in the model plant arabidopsis. Although an antioxidant response and a defense mechanism against several abiotic-stress conditions is activated by NO2-Ln and the ability of this molecule to release NO has also been reported, the precise mechanisms by which this nitro-fatty acid is able to induce these responses remain unknown. In this regard, future research should be focused on analyzing the electrophilic ability of NO2-FAs with specific plant-cell targets and their involvement in plant signaling. It is also important to take into account the capacity of NO2-Ln to release NO as well as the further implications of this molecule in a wide range of actions in plant physiology. Moreover, other mechanisms involving NO2-FAs could be considered which will hopefully offer insight into how NO2-Ln signaling regulates the important antioxidant and defense mechanisms that this molecule is able to set up. Finally, current mass spectrometry approaches have achieved a higher sensitivity allowing the routine and suitable level detection of these important signaling molecules. Therefore and in view of this landscape, NO2-FAs could be considered as new key mediators of NO metabolism in both physiological and stress conditions.

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