Nitric oxide: A radical molecule with potential biotechnological applications in fruit ripening

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Abstract

Nitric oxide (NO) is a short-life and free radical molecule involved in a wide range of cellular, physiological and stressful processes in higher plants. In recent years it has been observed that exogenous NO application can palliate adverse damages against abiotic and biotic stresses. Conversely, there is accumulating information indicating that endogenous NO participates significantly in the mechanism of modulation of the ripening in climacteric and non-climacteric fruits. Even more, when NO is exogenously applied, it can mediate beneficial effects during ripening and postharvest storage being one of the main effects the increase of antioxidant systems. Consequently, NO could be a promising biotechnological tool to improve crops through ameliorating nutritional indexes and to alleviate damages during fruit ripening and postharvest management. Thus, this approach should be complementary to previous strategies to allow preserving the quality and healthiness of fruits with a view of enhancing their added value. The present mini-review aims to provide an overview of NO biochemistry in plants and updated information on the relevance of NO in fruit ripening and postharvest stages with a view to its biotechnological applications.

Keywords: Antioxidants, fruit ripening, nitric oxide, postharvest, fruit quality

1. Overview of NO biochemistry in higher plants

Nitric oxide (NO ; NO hereinafter) is a free radical which is endogenously generated in plant cells as a common metabolite. Although its enzymatic source in higher plants is still under debate in the plant research community (Corpas and Barroso, 2016; Kolbert et al., 2019), there is no doubt that NO exerts regulatory function in a myriad of physiological and stress processes (Corpas and Barroso, 2015; Umbreen et al., 2018; Begara-Morales et al., 2018). NO acts either directly or through a family of derived molecules designated as reactive nitrogen species (RNS). These include nitrogen dioxide (NO_2), peroxynitrite (ONOO^-), and nitrosogluthathione (GSNO), among others (Begara-Morales et al., 2014; Corpas 2017; Takahashi and Morikawa, 2019; Staszek and Gniazdowska, 2020). The biochemistry of NO in plant cells has become very relevant since it can regulate protein functions throughout posttranslational modifications (PTMs), mainly nitration, *S*-nitrosation and metal nitrosylation. Besides, NO can also interact with other biomolecules including unsaturated fatty acids (FAs) and nucleic acids. **Figure 1A** provides a schematic model of the main interactions of NO with the different plant macromolecules, basically DNA, unsaturated fatty acids and proteins.

1.1. NO-derived PTMs

1.1.1. Protein nitration ($\text{NO}_2\text{Tyr-P}$)

Research on protein nitration has been mainly focused on the amino acid tyrosine (Tyr), although tryptophan (Trp) can also undergo this process (Yamakura and Ikeda, 2006). Tyrosine nitration involves the addition of a nitro group ($-\text{NO}_2$) to one of the two equivalent ortho carbons of the aromatic ring of Tyr residues. This may affect, in different degree, the structure, turnover and function of the target protein. Nitration is very selective because not all Tyr residues present in a protein are susceptible to be nitrated (Corpas et al., 2013a). In animal systems, two pathways have been proposed to mediate the protein nitration ($\text{NO}_2\text{Tyr-P}$): peroxynitrite-dependent and -independent mechanisms (Bartesaghi and Radi, 2018; Ferrer-Sueta et al. 2018). On one side, peroxynitrite (ONOO^-), as a nitrating molecule, is formed by the fast and spontaneous reaction between two radical molecules, nitric oxide (NO) and superoxide ($\text{O}_2^{\bullet-}$). On the other hand, the independent mechanism is mediated by the radical nitrogen dioxide (NO_2) which is generated by a reaction of hydrogen peroxide (H_2O_2) and nitrite (NO_2^-) in the presence of a heme-peroxidase (HPO) (**Fig. 1B**). These two mechanisms are not mutually exclusive, and depending on the circumstances of the cell, one could predominate over the other. Although in plant system there

is no doubt that protein nitration takes place endogenously under physiological and stress conditions (Corpas et al., 2008; Begara-Morales et al., 2013). To our knowledge, few specific studies are addressing which of these two mechanisms is the most prominent in each situation. Additionally, there are only some reports that through *in vitro* assays, have also shown the presence of nitration of either proteins or other non-protein peptides, by the two mentioned mechanisms (Sakihama et al. 2003; Corpas et al., 2013b).

On the other hand, NO₂Tyr-P is considered irreversible and the effect of nitration might be either the gain or loss of function of the affected protein, or even having no effect; however, the most frequent impact implies the loss of function (Begara-Morales et al., 2013, 2014; Corpas et al., 2013b; Castillo et al., 2015; Arasimowicz-Jelonek and Floryszak-Wieczorek, 2019; Palma et al., 2020). As a matter of fact, an increase in the content of protein Tyr nitration is considered a plausible marker of nitro-oxidative stress (Corpas et al., 2007).

1.1.2. Protein S-nitrosation (P-SNO)

Protein S-nitrosation, frequently called S-nitrosylation, involves the covalent attachment of an NO group to the thiol (–SH) side chain of cysteine (Cys) from either protein or small peptides. As in the nitration process, there are several possible mechanisms which allow the reaction of protein S-nitrosation (P-SNO) (Martínez-Ruiz and Lamas, 2004; Heinrich et al., 2013; Stomberski et al., 2019). The first mechanism involves the participation of the nitrosonium cation (NO⁺) which can be generated from a metal-dependent NO oxidation. This NO⁺ reacts with a thiolate anion (RS[–]) present in proteins or peptides to form the corresponding S-nitrosothiol (R-SNO). The second one implies the interaction between NO with a protein thiyl radical (RS[•]) to trigger the corresponding S-nitrosation. Another mechanism is by a process of transnitrosation where a NO group from an S-nitrosothiol (R-SNO), for example S-nitrosoglutathione, is transferred to another protein thiol (**Fig. 1C**).

Unlike protein nitration, S-nitrosation is a reversible process and it is considered a cellular switch which allows regulating the function of the target proteins. So far the number of identified S-nitrosated proteins in higher plants is increasing, thanks to the application of new (proteomic) approaches, and their regulation by S-nitrosation is better understood. Nevertheless, the specific effect of this PTM on a target protein needs to be analyzed because it has been shown that S-nitrosation can modulate either positively but also negatively the protein function. The reaction of NO with the thiol present in the tripeptide glutathione (GSH) generates nitrosoglutathione

(GSNO) which has been demonstrated to be of great relevance as a mechanism to extend the NO signaling properties inside of the cell/tissue since it can mediate the process of *S*-transnitrosation to proteins (Airaki et al., 2011; Broniowska et al., 2013; Corpas et al., 2013).

1.1.3. Protein metal nitrosylation

Protein metal nitrosylation involves the interaction between NO with transition metals (Fe or Cu) present in specific metalloproteins (Wasser et al., 2002; Zhang et al., 2016) such as hemoglobin, cytochrome *c* oxidase, guanylate cyclase and catalase (Brown 1995; Cooper 1999). However, thus far the information in plant system about this PTM is very limited, although it is plausible that, mirroring animal systems, plant heme-proteins such as the mitochondrial cytochrome *c* oxidase or the peroxisomal catalase can undergo metal nitrosylation considering that these organelles have an active NO metabolism (Kolbert et al., 2019; Corpas et al., 2020; Palma et al., 2020).

1.2. Nitro-fatty acids (NO₂-FAs)

NO-derived molecules such as [•]NO₂ and ONOO⁻ can react with unsaturated fatty acids through non-enzymatic reactions and generate nitro-fatty acids (NO₂-FAs) (Schopfer and Khoo, 2019). The presence of these molecules was initially reported in the animal system (O'Donnell et al., 1999), where they participate in diverse processes such as protection against cardiac ischemic injury or mediating anti-inflammatory signaling events (Cui et al., 2006). Years later, the presence of NO₂-FAs was demonstrated in plant systems. Specifically, it was demonstrated the presence of nitro-linolenic acid (NO₂-Ln) in *Arabidopsis* plants, where its content increased under several abiotic stresses (salinity, low temperature, wounding, and cadmium) through the induction of heat shock proteins (Mata-Pérez et al., 2016a). The relevance of NO₂-FAs resides also in their capacity to deliver NO to other sites (Mata-Pérez et al 2016b) and to interact with protein thiol groups to generate nitroalkylated proteins (Aranda et al., 2019), thus providing to them signaling capacity (Mata-Pérez et al., 2017). More recently, it has been shown that NO₂-oleic acid mediates reactive oxygen species (ROS) production through the activation of the NADPH oxidase in *Arabidopsis* (Arruebarrena et al., 2020). NO₂-oleic acid has been also found in *Brassica napus* seeds where it seems to participate in some germination steps (Vollár et al., 2020).

1.3. NO and nucleic acid nitration

Peroxynitrite has been shown to have the capacity to damage nucleic acids by the generation of both 8-oxoguanine (8-oxo-G) and 8-nitroguanine (8-NO₂-G), the latter being considered a reliable biomarker of nucleotides damage mediated by RNS (Szabó and Ohshima, 1997; Masuda et al., 2002; Ohshima et al., 2006). Thus, the presence of 8-NO₂-G is considered as a potential carcinogenic marker in diverse pathologies in animal systems (Kawanishi et al. 2017).

In plants, our knowledge about 8-NO₂-G is less advanced than in animal systems, and only a few recent studies have been focused in this area of NO metabolism (Chmielowska-Bąk et al., 2019; Arasimowicz-Jelonek and Floryszak-Wieczorek, 2019). Thus, it has been found that 8-NO₂-G in the RNA chains seems to affect the post-transcriptional gene expression regulating the programmed cell death during the avirulent plant-pathogen interaction in potato (Izbińska et al., 2018). On the other hand, RNA nitration has also been found to increase under physiological processes such as seed dormancy breakage in apple (Andryka-Dudek et al., 2019).

RNS can react with other purine nucleosides such as guanosine to generate 8-nitroguanosine. Phosphorylated guanosine is part of the cyclic guanosine monophosphate (cGMP) and the formation of 8-nitroguanosine cyclic monophosphate (8-nitro-cGMP) seems to affect signalling processes (Sawa et al., 2013). The formation of nitrated cGMP seems to be a promising new area of plant research (Arasimowicz-Jelonek and Floryszak-Wieczorek, 2019; Petřivalský and Luhová, 2020). Very recently, it has been found an accumulation of 8-nitroguanosine during the internal browning associated to oxidative stress via the Maillard reaction in sweet potato roots (Fukuoka et al., 2020).

2. Fruit ripening and endogenous NO metabolism

Fruit ripening is a physiological and genetically programmed process which is highly regulated because it involves severe biochemical, transcriptional and morphological modifications (Palma et al., 2015, 2018; Corpas et al., 2018; Yan et al., 2018; González-Gordo et al., 2019, 2020). Fruits can be categorized by different criteria, so they are classified as either dry/fleshy (considering if their water content is below/above 50%, respectively), dehiscent/indehiscent (according to the opening mechanism of fruits and seed dispersion; Mühlhausen et al., 2013; Ballester and Ferrándiz, 2017) or climacteric/non-climacteric. In this latter case, climacteric fruits such as apples, bananas, melon, peaches or tomatoes are characterized by an increase of ethylene production accompanied by a rise in cellular respiration during their ripening. On the contrary, in non-climacteric fruits such as strawberries, grapes, oranges, peppers, the ripening process is

independent of the ethylene production (Cherian et al., 2014), where the rates of ethylene production and respiration are very low (Hou et al., 2018; Reis et al., 2020).

Pioneer Leshem's studies indicated that NO was involved in the mechanism of fruit ripening and it had an antagonistic effect to ethylene (Leshem et al., 1998; Leshem and Pinchasov, 2000; Leshem 2000). Accordingly, the exogenous application of NO to banana fruits extended their postharvest life (Ku et al., 2000; Wills et al., 2000; Leshem et al., 2001; Wills et al., 2003). Despite these promising early reports on the interaction of NO in the fruit physiology, only few studies have been focused on the endogenous metabolism of NO during ripening. The majority of the research has been dedicated in its potential biotechnological application either to extend the postharvest life of fruits or to avoid associated damages.

Recently, using the non-climacteric sweet pepper (*Capsicum annuum* L.) fruit as a model, the metabolism of endogenous NO and related species during ripening has been investigated. As a result, the endogenous NO content decreased significantly during pepper ripening, whereas the SNOs level and protein nitration increased (Chaki et al., 2015; Palma et al., 2018). Additionally, it was reported that the activity, protein content and gene expression of the GSNO reductase (GSNOR) underwent a decay as pepper fruits ripen, and this was accompanied but an increase of *S*-nitrosated proteins (Rodríguez-Ruiz et al., 2017a). Furthermore, new experimental pieces of evidence provided deeper insights into the close relationship between NO, ROS, hydrogen sulfide (H₂S) and NADPH-generating systems in the fruit physiology. Accordingly, in pepper fruits, the peroxisomal H₂O₂-regulating catalase activity was shown to be down-regulated by both nitration and *S*-nitrosation (Rodríguez-Ruiz et al., 2019), but also by H₂S (Corpas et al., 2019a; Palma et al., 2020). On the other hand, the O₂^{•-}-generating NADPH oxidase activity, also designated as respiratory burst oxidase homolog (Rboh), which is up-regulated during sweet pepper fruit ripening was also inhibited by nitration and *S*-nitrosation (Chu-Puga et al., 2019). Interestingly, none of the four superoxide dismutase (SOD) isozymes identified in sweet pepper seemed to be modulated during ripening and were also unaffected by NO (González-Gordo et al., 2020).

NADPH is a key cofactor required for cell growth, cell proliferation and detoxification which participates in the redox status of the cells. Accumulating data indicate that NADPH is needed to maintain multiple metabolic pathways involved in the natural fruit ripening being considered as a quality footprint of crops (Aghdam et al., 2020). In sweet pepper fruit, some of the main NADPH-generating enzymes are differentially modulated during ripening. Thus, whereas the

NADP-isocitrate dehydrogenase and the glucose-6-phosphate dehydrogenase (G6PDH) activities diminished in ripe fruits, the NADP-malic enzyme (NADP-ME) and the 6-phosphogluconate dehydrogenase (6PGDH) increased. Furthermore, these enzymes were also differentially regulated by NO and H₂S in pepper fruits at different ripening stages. The reported data indicate that whilst the activity of the two components of the oxidative pentose phosphate pathway (G6PDH and 6PGDH) were unaffected by these two molecules, the NADP-ICDH and NADP-ME activities were inhibited in ripe pepper fruits (Muñoz-Vargas et al. 2018, 2019). It has been reported that H₂S can regulate the activity of proteins by a PTM designated persulfidation which affect the thiol group of Cys as the *S*-nitrosation does. Consequently, persulfidation and *S*-nitrosation co-exist in cells, thus indicating that these two molecules, NO and H₂S may have synergistic or antagonistic functions competing for Cys thiols (Corpas et al., 2019b, 2020). This is one of the hot issue in the current plant signaling research.

3. Nitric oxide and ethylene interactions in climacteric fruits

Such as it has been mentioned above, the ripening of climacteric fruits is promoted by the phytohormone ethylene (Liu et al. 2015; Li et al., 2019). NO exerts regulatory functions in the activity and gene expression of the actors involved in the ethylene signalling pathway and the quality of post-harvest fruits (Leshem and Pinchasov, 2000; Lindermayr et al., 2006; Eum et al., 2009; Deng et al., 2013; Hong et al., 2014; Mukherjee, 2019; Zuccarelli et al., 2020). Nevertheless, fruit ripening seems to be also regulated by other phytohormones including auxin, abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) (Zhang et al., 2009; McAtee et al., 2013; Kumar et al., 2014; Palma et al 2019). However, the mechanism of interaction among these phytohormones with NO during ripening at the endogenous level is quite limited, and the available information has been obtained by the exogenous application of NO in combination with some of these signalling molecules such as SA or methyl jasmonate (Hong et al., 2014; Guo et al., 2014; Vithana et al., 2019).

Figure 2 shows a schematic model of ethylene biosynthesis. Starting from the amino acid methionine (Met), *S*-adenosylmethionine (SAM) is synthesized by the enzyme *S*-adenosylmethionine synthetase (SAMS) which requires ATP to be carried out. Then, SAM is transformed to ethylene by the action of two enzymes: 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO). These two enzymes are highly regulated at the transcriptional and post-transcriptional level and, using different plant models, it has been

demonstrated that NO exerts a down-regulation of both ACS and ACO, although the molecular mechanism has not been yet deciphered (Zhu and Zhou, 2007; Cheng et al., 2009; Eum et al., 2009; Melo et al., 2016; Asgher et al., 2017; Kolbert et al., 2019; Palma et al., 2019). SAMS, also called methionine adenosyltransferase (MAT), has been reported to be down-regulated by S-nitrosation (Lindermayr et al., 2006). Whereas in the case of ACC oxidase, an iron-containing enzyme, NO seems to be bound through a metal-nitrosylation process allowing the formation of a stable ternary complex (ACC-ACO-NO) which blocks the ethylene biosynthesis (Manjunatha et al., 2012; Singh and Bhatla, 2018). Accordingly, when talking on ripening in climacteric fruits ethylene and NO operate as coin head and tail which finally influence their lifespan and quality.

4. NO regulates phenylpropanoid metabolism during fruit ripening

Phenylpropanoid pathway generates a wide range of metabolites involved in the biosynthesis of lignin, necessary for the firmness of some fruits, or flavonoids such as anthocyanins which, in the case of fruits, affect pigmentation and disease resistance (Singh et al. 2010; Wang et al., 2020). In the case of pepper fruits, phenylpropanoids are also precursors of capsaicin and other capsaicinoids, alkaloids exclusive for *Capsicum* species, which provides to their fruits the typical pungency trait (Kang et al., 2005; Nuñez-Palenius and Ochoa-Alejo, 2005; Palma et al., 2020).

An early report indicated that the exogenous NO application, using the sodium nitroprusside (SNP) donor in Arabidopsis and maize plants, protected from UV-B by up-regulating the gene expression of chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*) and flavonol synthase 1 (*FLS1*), enzymes involved in the phenylpropanoid pathway (Tossi et al., 2011). Later, Li and colleagues (2017) showed that in peach fruit exposed to NO gas there is a stimulation of the phenylpropanoid pathway due to an increase in the activity and gene expression of several key enzymes including phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase, 4-coumaroyl-CoA ligase, CHS and CHI. The consequence of this NO treatment was a higher content of total phenolics, anthocyanins and lignin, as a result improving disease resistance in this climacteric fruit. A similar response has been described in other climacteric fruits, for example in apples treated with SNP an increase in the content of lignin, total phenolic compounds and flavonoids were triggered, providing an enhanced disease resistance to the fungus *Penicillium expansum* (Ge et al. 2019a). In the case of the blueberry fruits treated with SNP, it was also found a stimulation of the phenylpropanoid pathway which was accompanied by a significant increase of antioxidant enzymes including SOD, ascorbate

peroxidase (APX) and glutathione reductase (GR). This situation allowed maintaining the fruit quality and antioxidant ability during postharvest storage (Ge et al. 2019b). On the other hand, the exogenous application of SNP on goji (*Lycium barbarum* L) fruits, which belong to the non-climacteric group, provoked an attenuation of coloration, due to anthocyanin and proanthocyanidin, by antagonizing the ABA action that caused a delay of the fruit ripening (Li et al., 2020). The application of exogenous NO to hot pepper (non-climacteric), with high capsaicin levels, might be a good strategy to amply our knowledge on the interaction of this radical gas with the phenylpropanoids' metabolism. All these data support that NO exerts a regulatory function on the phenylpropanoid pathway, which indicates that NO is able to influence the organoleptic and nutritional features of fruits.

5. Biotechnological benefits of the exogenous NO application to fruits

From an anthropogenic viewpoint, fruits are nutritional targets as essential sources of vitamins or antioxidants, and this is why their consumption is considered very healthy. For this reason, during their production and postharvest management, the horticultural industry seeks to obtain fruits with healthy features, keeping their texture, external appearance, nutritive value, aromas and flavors attractive to the human palate. Additionally, during postharvest storage, besides keeping the fruit quality, the new technology and marketing perspectives have sought for additional goals which involve to maintain food safety and to reduce losses between harvest and consumption (Boeckx et al., 2019; Brizzolara et al., 2020). Furthermore, the search of new diagnostic tools is still looking for the abovementioned fruit safety purposes (Kumar and Thakur, 2018).

Exogenous NO applications have been frequently used as a strategy to study plant metabolism, especially under abiotic stress conditions such as heavy metals, salinity, drought, and extreme temperature (Manai et al., 2014; Kharbech et al., 2017; Hasanuzzaman et al., 2018; Sohag et al., 2020). So far the application of NO has been basically done for academic research using different types of donors such as the diethylenetriamine-nitric oxide (DETANO), sodium nitroprusside (SNP), *S*-nitrosoglutathione (GSNO) or NO gas. The most common NO donor used in plant research when it is applied to the whole plant is SNP, because it is economically cheaper in comparison with other compounds such as NONOate (diethylamine NONOate or spermine NONOate) or nitrosothiols (*S*-nitroso-*N*-acetylpenicillamine or GSNO), which are generally used for *in vitro* assays. It should be mentioned that NO gas in ambient air condition is oxidized to

generate the radical nitrogen dioxide ($\cdot\text{NO}_2$) which is a pollutant dark-brown gas according to the following reaction: $2 \cdot\text{NO} (\text{g}) + \text{O}_2 (\text{g}) \rightarrow 2 \cdot\text{NO}_2 (\text{g})$. To avoid its formation during the application of NO gas several options have been used such as the direct injection of NO gas into the containers which house plants, with nitrogen (N_2) gas as a carrier (Zhu et al., 2006; Zhao et al., 2019, 2020). Another option is to provide the NO gas throughout a system with include two Büchner flasks placed in series: the first flask contains copper wires and a saturated HNO_3 solution. Both chemicals react rendering NO and $\cdot\text{NO}_2$. Both gases are driven into a second flask containing a NaOH solution which traps the generated $\cdot\text{NO}_2$ according to the following reaction: $2 \cdot\text{NO}_2 + 2 \text{NaOH} \rightarrow \text{NaNO}_2 + \text{NaNO}_3 + \text{H}_2\text{O}$. Then, the NO is released to the chamber where plant material is exposed to the gas (Palma et al., 2018).

Fruits like apple, banana, citrus, grape, mango or tomato, among others, can undergo different damages during postharvest and storage such as compositional changes, water loss and shrinkage, excessive softening, chilling injury, decay or bacteria/insect attack. Several biotechnological protocols are ordinarily applied to avoid those effects, like appropriated packaging procedures, low and high-temperature treatments, modified atmosphere storage (low CO_2), as well as the use of some chemicals such as sulfur dioxide, sodium hypochlorite, and potassium bicarbonate (Gorny 2001). Lately, new compounds have started to be studied to reduce or palliate those undesirable symptoms. Some of them include chitosan (Romanazzi et al., 2018), melatonin (Wang et al., 2020; Zhang et al., 2020), hydrogen sulfide (Geng et al., 2019; Corpas and Palma et al., 2020) and also NO (Corpas and Palma, 2018). **Table 1** summarizes some representative examples of the beneficial effects exerted by the application of exogenous NO in climacteric and non-climacteric fruits. It should be remarked that in all cases the mechanism to apply the NO, its concentration, time of exposition or stage of fruit development was different in each case, according to the peculiar features of each fruit.

A common characteristic observed after NO application to fruits is the alteration of ROS metabolism which stimulates enzymatic and non-enzymatic antioxidant systems. Consequently, this can palliate oxidative damage associated with postharvest and storage (**Fig. 3**). However, NO can also modulate the ripening process. For example, in pepper fruits, the application of NO gas during the transition from the immature (green) to ripe (red) stages has been shown to modulate positively the ascorbate content due to upregulation of the galactono-1,4-lactone dehydrogenase (Rodríguez-Ruiz et al., 2017b). This enzyme catalyzes the last step of the ascorbate biosynthetic

pathway. A deeper analysis of the antioxidant catalase demonstrated that this enzyme is modulated by RNS but also by ROS (Rodríguez-Ruiz et al., 2019a; Palma et al., 2020). Transcriptomic and biochemical analyses of pepper fruits reveal that NO treatment resulted in differential expression of 498 genes framed within functional categories which involve ROS, RNS and lipid metabolism (González-Gordo et al., 2019). Furthermore, the biochemical analysis showed that NO treatment triggered significant changes in fatty acid profiling, glutathione and proline contents, and the extent of lipid peroxidation, as well as increases in the activity of APX and lipoxygenase (LOX). Complementary studies indicate that the observed $O_2^{\cdot-}$ generation by an NADPH-dependent oxidase system, which usually increased during ripening, was negatively modulated by NO (González-Gordo et al., 2020).

Additionally, NO can implement the effect of other compounds. For example, the application of NO improves the effect of 1-methylcyclopropene (1-MCP) to extend the postharvest life in blueberry (Gergoff et al., 2017) and tomato (Steelheart et al., 2019) fruits. Likewise, the increased resistance triggered by ABA to cold-induced oxidative stress seems to be mediated by NO in peaches during cold storage since the endogenous NO synthesis is induced by the nitrate reductase pathway (Tian et al. 2020). Likewise, in peach fruit storage a low temperature, exogenous NO promotes the emission of volatile organic compounds (VOCs) such as benzaldehyde, nonanal, D-limonene, D-camphor and geranylacetone. This palliates the inhibitory effect of VOCs provoked by low temperature (Cai et al 2020). A similar observation has been also described in parallel studies where exogenous NO applied to peach fruits stimulated the sphingolipid metabolism during the postharvest storage a low temperature (Huang et al., 2020). This is very significant since sphingolipids are important in regulating cell senescence and response to cold stress.

Overall, considering the data given above, the treatment of fruits with exogenous NO seems to be a promising biotechnological tool to boost their nutritional value based on the antioxidants, vitamins and mineral contents. Besides, this approach can also influence the contents of the many secondary metabolites reported in plants with pharmacological and therapeutic uses. Therefore, fruits treated with NO could have an added value as a potential functional food.

6. Conclusion and future perspective

Nitric oxide is a free radical with signaling properties which interacts with a great diversity of biomolecules (proteins, fatty acids and nucleic acids) affecting their functions. During the last 20 years, significant advance has been made in the mechanism of functions of endogenous NO in plant cells, although there are still some gaps such as the identification of its specific enzymatic sources in each subcellular compartment and organ. However, many studies have shown that exogenous NO can exert beneficial effects at different levels (Marvasi, 2017; Rodríguez-Ruiz et al., 2019b) and modulate processes associated with the fruit physiology. More recently, accumulating evidence indicates that the gasotransmitter H₂S also exerts signaling functions including the process of fruit ripening. Understanding the intimate mechanisms by which these processes are regulated and the interaction NO with other molecules such as phytohormones or H₂S in fruits will be the target of upcoming investigations to get healthy products with nutraceutical potentialities.

An additional issue that should be addressed is the transit from academic research to crops and post-harvest improvement. The experimental designs followed to understand the mechanism by which NO (and other gasotransmitters) may affect the nutritional value of fruits are not easily applicable to crops. Seeking for biotechnological alternatives, environmental and agronomical friendly and with low cost should drive our targets in the future. And last, but not least, the search of strategies which promote the endogenous NO (and other gasotransmitters) production in fruits should not be ruled out.

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Table 1. Exogenous application of NO on fruit and its beneficial effects. ABA, abscisic acid. APX, ascorbate peroxidase. CAT, catalase. GR, glutathione reductase. GST, glutathione-S-transferase. H₂S, hydrogen sulfide. IP₃, inositol 1,4,5-trisphosphate. LOX, lipoxygenase. MSR, methionine sulfoxide reductase. NR, nitrate reductase. POD, peroxidase. SOD, superoxide dismutase. SUMO, small ubiquitin-like modifier

Fruit	Effects of exogenous NO	Reference
Climacteric (ripening is associated to ethylene production)		
Apple (<i>Malus x domestica</i> Borkh. cv. 'Granny Smith')	Inhibits browning in apple slices ⁽¹⁾	Pristijono et al., 2008
Papaya (<i>Carica papaya</i> L. cv. 'Sui you 2')	Delays softening and ripening ⁽²⁾	Guo et al., 2014
Peach (<i>Prunus persica</i> L. cv. 'Xiahui NO.5')	Up-regulates the abundance of SOD. Increases disease resistance ⁽²⁾	Kang et al., 2016; Li et al., 2017
Peach (<i>Prunus persica</i> cv. (L.) Batsch, cv. "Xintaihong")	Regulates H ₂ S metabolism and the fruit quality during cold storage	Geng et al., 2019
Peach (<i>Prunus persica</i> Batsch cv. Jinqiuhongmi)	Increases the activity of SOD, POD, CAT, APX, GST and GR. Increases expression of MSR and SUMO. Inhibits activity and expression of LOX and PLD. IP ₃ is involved in NO-enhanced chilling tolerance ⁽³⁾	Jiao et al., 2019
Peach (<i>Prunus persica</i> L. Batsch cv. Xiahui 6)	Promotes the emission of volatile organic compounds. Alleviates chilling injury. Promotes the recovery of respiration rate and ethylene production during shelf life ⁽²⁾	Cai et al., 2020
Peach (<i>Prunus persica</i> L. Batsch, cv. Feicheng)	Prevents chilling injury. Regulates sphingolipid metabolism during low temperature storage.	Zhu et al., 2010; Huang et al., 2020
Peach s (<i>Prunus persica</i> L. Batsch, cv Xintaihong)	In combination with ABA regulates biosynthesis of endogenous NO via the NR pathway during fruit storage	Tian et al. 2020
Tomato (<i>Solanum lycopersicum</i> L. cv. Ailsa Craig, AC)	Delays fruit ripening and increase activity of antioxidant enzymes ⁽³⁾	Lai et al., 2011
Tomato (<i>Solanum lycopersicum</i> L. cv 'Elpida')	NO ⁽⁴⁾ in combination with 1-methylcyclopropene extends postharvest life	Steelhearta et al., 2019
Tomato (<i>Solanum lycopersicum</i> L. cv. Micro-Tom)	Increases in the content of total ascorbate (25%) and flavonoids (60%) ⁽²⁾	Zuccarelli et al., 2020
Kiwi (<i>Actinidia chinensis</i> L. Planch cv Xuxiang)	Extends postharvest life and enhances antioxidant activity ⁽²⁾	Zhu et al., 2010
Non-Climacteric (ripening is not-associated to ethylene production)		
Strawberry (<i>Fragaria x ananassa</i> Duch.)	Extends postharvest life ⁽³⁾	Wills et al., 2007; Zhu & Zhou, 2007
Sweet pepper (<i>Capsicum annuum</i> L. cv. Melchor)	Delays fruit ripening ⁽²⁾	Chaki et al., 2015;

Sweet pepper (<i>Capsicum annuum</i> L. cv. Melchor)	Increases ascorbate content ⁽²⁾	Rodríguez-Ruiz et al., 2017a
Orange (<i>Citrus sinensis</i> L. Osbeck cv. Valencia)	Disease resistance to the fungus <i>Colletotrichum gloeosporioides</i> ⁽³⁾	Zhou et al., 2016
Bayberry (<i>Myrica rubra</i> Sieb. & Zucc. cv. Dongkui)	Decreases in firmness and increases the activity of SOD, CAT and APX ⁽²⁾	Wu et al., 2012
Blueberry (<i>Vaccinium</i> spp.)	NO ⁽⁴⁾ in combination with 1-methylcyclopropene extends postharvest life	Gergoff et al., 2017
Jujube (<i>Zizyphus jujuba</i> Mill. cv. Dongzao)	Enhances the ROS scavenging capacity, retards cell wall degradation and delays the senescence symptoms ⁽²⁾	Zhao et al., 2019, 2020

⁽¹⁾Diethylenetriamine-nitric oxide (DETANO) as NO donor

⁽²⁾NO gas

⁽³⁾Sodium nitroprusside (SNP) as NO donor

⁽⁴⁾S-nitrosglutathione

Figure 1. (A) Main modifications mediated by nitric oxide (NO) which affect DNA, fatty acids, proteins and peptides. (B) Possible mechanisms of protein tyrosine nitration. (C) Possible mechanisms of protein S-nitrosation. HPO, heme-peroxidase. See text for more details.

Figure 2. Model of NO action on ethylene biosynthesis.

The biosynthesis of ethylene from methionine involves three enzymes designated S-adenosylmethionine synthetase (SAMS), 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO). NO can exert a down-regulation on each of these enzymes. However, only in the case of SAMS it has been shown that it occurs by S-nitrosation.

Figure 3. Working model of the main effect of the exogenous application of nitric oxide (NO) on climacteric and non-climacteric fruits.