Review

**Melatonin and reactive oxygen and nitrogen species: a model for the plant redox network**

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**Running title:** Melatonin in plant redox network

Received: July 24, 2019; Accepted: August 26, 2019

**ABSTRACT**

Melatonin (N-acetyl-5-methoxytryptamine) was discovered in plants in 1995; since then numerous functions have been attributed to this molecule in vascular plants. In addition to its recognized role as a universal antioxidant, other relevant functions have been studied in plants such as its rhizogenic- and vegetative-growth effects, protection against leaf senescence and influences on photosynthesis and on the stomatal apparatus. Also, melatonin has a protective role in stress situations (biotic and abiotic), acting as an osmoregulation and a metabolic corrector when confronted with different stresses. One of the most outstanding aspects is the involvement of melatonin as a multi-signaling molecule in plants. The dual roles of melatonin in physiological stress situations involve both its direct action (free of receptor action) as an antioxidant and its role as a regulator of gene expression. Its relationship with central elements of the plant redox network such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the regulation of relevant elements is discussed. All recent data on melatonin are incorporated to present an updated model, where the balance between ROS and RNS, and between these and melatonin is a regulatory key center in the responses.

**Keywords:** Melatonin, NO, plant stress, redox network, ROS, RNS.

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1. **INTRODUCTION**

Melatonin (N-acetyl-5-methoxytryptamine) was discovered in 1958 in the pineal gland of cow by Lerner *et al.* (1). This isolated active factor lightens skin color in frogs, tadpoles, toads and certain fish, but not in mammals. Melatonin is of particular importance as a chronobiological hormone, acting as a signal of darkness that provides information to the brain and peripheral organs (2). In 1995, three independent research groups published the unequivocal identification of melatonin in plants (3-5). This fact caused uncertainty since the presence of an animal hormone in plant tissues was unexpected. In the first few years of its discovery in plants, the research focused on physiological roles similar to those that melatonin had in animals, such as an antioxidant mainly. A few years later, many of the actions that melatonin has in plants such as germination, growth and rooting promoter, and also as a foliar senescence retardant were demonstrated, mainly in stress conditions (6). Also, the melatonin biosynthesis route in plants has been completed with great accuracy, thanks in particular to the works of K. Back and J. Kong in rice and *Arabidopsis* plants (7-20).
The objective of this review is to give a global vision of the relationship between ROS, RNS and melatonin within the redox network of plants. An updated model in which both direct and indirect interactions between these three elements is presented, highlighting the aspects of gene regulation controlled by melatonin in stress conditions.

2. STRESS & ROS/RNS: ABIOTIC & BIOTIC STRESSES

In general, stressors, either biotic or abiotic, are the factors that most affect the normal physiological behavior of plants and also productivity in crops. Under stress situations the equilibrium between oxidant and antioxidant chemical species in the cell is lost, altering redox homeostasis, and giving rise to an excessive accumulation of ROS. The set of physiological alterations caused and their harmful/deleterious effects on the cells is known as oxidative stress (21-24). Interestingly, the cell uses some of these ROS as signaling agents that trigger a whole set of responses, and thus deal with the stressful agent(s) (25-28). Thus, ROS can play a dual role in plant cells: at low levels they can act as signaling agents inducing a positive response to recover redox homeostasis. At high endogenous levels, ROS are toxic and harmful to the cells, provoking an increase in the cellular oxidative status which results in severe damage to proteins, lipids and even nucleic acids. These severe consequences can be avoided or, at least, mitigated due to the existence of a set of antioxidant agents (metabolites and enzymes) that appear in the cell as an anti-stress response, neutralizing and eliminating ROS overproduction (see below).

Similar to ROS, RNS are a set of radical and non-radical species that are generated in the cell during stress conditions, in a process known as nitrosative stress (29, 30). In the same way as ROS, RNS also present the dual aspects: on the one hand at high levels they are harmful to the cell due to their radical and/or highly oxidative nature, and, on the other hand, some of them are capable of acting as signaling molecules, regulating important physiological processes. Thus, ROS and RNS seem to act by regulating processes such as seed germination, plant growth, organogenesis, reproduction, senescence, and also responses to abiotic stressors (drought, salinity, cold, heat, UV radiation, chemical agents, among others) and biotic stressors (28, 31-36).

3. METABOLISM OF ROS AND RNS

ROS are mainly generated by two chemical processes. The first is the electron transfer (between 1 and 3 electrons) to oxygen, resulting in the formation of superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) or hydroxyl radical (·OH). The second of these is the transfer of energy to molecular oxygen (O$_2$), leading to the formation of singlet oxygen ($^1$O$_2$) (37). Some characteristics of these ROS are shown in Table 1.

Several RNS have been described in plants. The most relevant and some of their characteristics are presented in Table 2. The most interesting ones are the radical species nitric oxide (·NO) and nitrogen dioxide (·NO$_2$) and the non-radical species peroxynitrite (ONOO$^-$) and S-nitrosglutathione (GSNO). Other RNS are: nitroxyl anion, nitrosonium cation, nitrate, nitrous acid, dinitrogen trioxide, dinitrogen tetroxide and nitryl chloride (37). Tables 1 and 2 also summarize some relevant aspects of these radical species such as their half-life which is usually very short and the main generator system and its localization in the plant cell. Of the eight chemical species that are presented, only three are generated from enzymatic reactions (O$_2^-$, H$_2$O$_2$ and ·NO), while the rest are generated in chemical reactions, without any biological catalyst (37).
Table 1. Characteristics of the main ROS species in plants.

<table>
<thead>
<tr>
<th>Specie (name)</th>
<th>Half-life</th>
<th>Main generators</th>
<th>and localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mathrm{O_2}^-$ (superoxide anion)</td>
<td>1-4 µs</td>
<td>RbOHs, Ferredoxin and ETC mitochondrial ETC PSI, PSII XAO</td>
<td>Apoplast</td>
</tr>
<tr>
<td>$\mathrm{H_2O_2}$ (hydrogen peroxide)</td>
<td>&gt; 1 ms</td>
<td>RbOHs, PAO, DAO, XAO, GOX, GSO, ASO, GPX, SOD, OXO</td>
<td>Apoplast, Mitochondria, Peroxisome</td>
</tr>
<tr>
<td>$\cdot$OH (hydroxyl radical)</td>
<td>1 ns</td>
<td>Fenton’s reaction from $\mathrm{H_2O_2}$ ($\mathrm{Fe}^{2+}$)</td>
<td>Anywhere in the cell</td>
</tr>
<tr>
<td>$^1\mathrm{O_2}$ (singlet oxygen)</td>
<td>4 µs</td>
<td>Chlorophyll triplet state LPO</td>
<td>Chloroplast, Nuclei</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the main RNS species in plants.

<table>
<thead>
<tr>
<th>Specie (name)</th>
<th>Half-life</th>
<th>Main generators</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\cdot$NO (nitric oxide)</td>
<td>&lt; 1 µs</td>
<td>Nitrate reductase (NR)</td>
<td>Chloroplast, Mitochondria, Peroxisome</td>
</tr>
<tr>
<td></td>
<td>~ 1 h</td>
<td>Nitrite reductase (NiR)</td>
<td>Plasma membrane-bound protein (Ni-NOR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peroxidase synthase (NOS-like enzyme)</td>
<td>Chloroplast, Mitochondria, Peroxisome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peroxidase (XOR)</td>
<td>Hydroxylamines and polyamines</td>
</tr>
<tr>
<td>ONOOO$^-$ (peroxynitrite)</td>
<td>10-20 ms</td>
<td>From $\cdot$NO$^+$, $\mathrm{O_2}^-$</td>
<td>Plasma membrane Apoplast, Cytosol</td>
</tr>
<tr>
<td>$\cdot$NO$_2$ (nitrogen dioxide)</td>
<td>40-70 s</td>
<td>From ONOOO$^-$ + H$^+$</td>
<td>Apoplast</td>
</tr>
<tr>
<td></td>
<td></td>
<td>From ONOOO$^-$ + CO$_2$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>From $\cdot$NO + $\mathrm{O_2}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>From $\cdot$NO + $\mathrm{O}_3$</td>
<td></td>
</tr>
<tr>
<td>GSNO (S-nitrosoglutathione)</td>
<td>8-12 min</td>
<td>From $\cdot$NO + GSH</td>
<td></td>
</tr>
</tbody>
</table>

ROS and RNS can be neutralized or scavenged by enzymatic and/or non-enzymatic systems (Table 3). Only $\mathrm{O_2}^-$ and $\mathrm{H_2O_2}$ can be catabolized by enzymes; by superoxide dismutases (SOD) for $\mathrm{O_2}^-$ and by catalases and peroxidases mainly for $\mathrm{H_2O_2}$. These last two ROS and the other
ROS and RNS can be scavenged by a very diverse set of antioxidant compounds which react with ROS and RNS by several complex mechanisms resulting in the neutralization of these reactive species (28, 37-39) (Table 3).

Table 3. Main scavengers of ROS and RNS species in plants.

<table>
<thead>
<tr>
<th>Specie (name)</th>
<th>Scavengers Enzymatic</th>
<th>Non-enzymatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2^-$ (superoxide anion)</td>
<td>SOD</td>
<td>Polyphenols, ASA, GSH, cys</td>
</tr>
<tr>
<td>H$_2$O$_2$ (hydrogen peroxide)</td>
<td>CAT, APX, GPX, GST, PER, PRX</td>
<td>ASA, GSH, Flavonoids, Polyamines</td>
</tr>
<tr>
<td>·OH (hydroxyl radical)</td>
<td></td>
<td>Flavonoids, proline, ASA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GSH, melatonin, sugars</td>
</tr>
<tr>
<td>¹O$_2$ (singlet oxygen)</td>
<td></td>
<td>Carotenoids, Tocopherols, Plastoquinone</td>
</tr>
<tr>
<td>·NO (nitric oxide)</td>
<td></td>
<td>O$_2^-$, O$_2$, Fe$^{2+}$, Flavonoids, ASA, Melatonin</td>
</tr>
<tr>
<td>ONOO$^-$ (peroxynitrite)</td>
<td></td>
<td>Thiols, Zn-SH groups, Iron/sulfur centers, ·OH/·NO$_2$/CO$_2$, Flavonoids, Phenolic acids, GSH, ASA, [H$^+$], Tryptophan, Melatonin (and related), PUFA, LDL, DNA (guanine)</td>
</tr>
<tr>
<td>·NO$_2$ (nitrogen dioxide)</td>
<td></td>
<td>·NO, Tocopherols</td>
</tr>
<tr>
<td>GSNO (S-nitrosoglutathione)</td>
<td></td>
<td>Similar to ONOO$^-$</td>
</tr>
</tbody>
</table>

4. MELATONIN AND ROS/RNS

Melatonin can act as an excellent antioxidant at cellular level. There are numerous data about the antioxidative role that melatonin exerts against several ROS/RNS and other oxidative agents. From the first experiments conducted by Reiter’s group in 1993, which demonstrated the in vitro scavenging efficacy of melatonin against ·OH (40-42), up to recent tests in which melatonin is presented as an excellent treatment against some herbicides and toxic compounds (Table 4), a plethora of reports on the important role of melatonin in multiple biochemical, physiological and pathological situations have been published. Melatonin acts against radical species through two major chemical mechanisms including single electron transfer and hydrogen transfer, although lesser known ones are possible (29, 43-47).

Table 4 shows a compilation of ROS/RNS and other harmful chemical species of biological relevance in both plants and animals that are susceptible to being neutralized by melatonin. The calculation of the efficiency of melatonin as a scavenger uses the data of Galano et al. (2011) comparing their respective second rate constants, log ($k$) (44). As noted, melatonin exhibits its
greatest scavenging capacity against ·OH and ·NO, ·NO₂, ·N₃ and ONOO⁻. Compared with other scavengers, melatonin has higher ·OH scavenging activity than mannitol and glutathione, and an IC₅₀ ~6-times lower than glutathione and ~13-times lower than mannitol (40). As regards H₂O₂, some controversy seems to exist since some authors attribute the scavenging ability of melatonin to hydrogen peroxide acting as an intermediation due to the presence of transition metals at very low levels in the reaction, thus acting as an inhibitor of the Fenton reaction (48, 49). Both in animal and plant cells, melatonin minimizes the toxic and harmful effects of several toxins, drugs and herbicides. Some examples appear in Table 4. In general, two actions seem to occur: a direct action of melatonin acting on the extra production of ROS/RNS caused by the foreign substance, and an indirect action that activates the gene expression of antioxidant enzymes such as SOD, catalases, ascorbate-, glutathione- and haloperoxidases, glutathione reductases, glutathione synthases, glutathione S-transferases, ascorbate oxidases, monodehydro- and dehydroascorbate reductases, peroxiredoxins, thioredoxins, etc.; all aimed at minimizing the toxic action of the substance (29, 50). The detoxifying action of the foreign substance mediated by melatonin for some drugs has also been described. A similar mechanism may explain the optimal results in melatonin-treated plants against metal toxicity such as Cd, Zn, Vn, Cu, Fe, Al, Pb, Ni, Co, among others (50-52). Melatonin has been seen to behave in plants as a similar way in animals (53).

Table 4. Comparative efficiency of melatonin as scavenger of different ROS, RNS and other toxics.

<table>
<thead>
<tr>
<th>Chemical specie</th>
<th>Relative efficiency log(k)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS &amp; RNS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂⁻</td>
<td>4</td>
<td>(44)</td>
</tr>
<tr>
<td>·OH</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>·NO</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>·NO₂</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>·N₃</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>ONOO⁻</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>LOO⁻</td>
<td>~0.2</td>
<td></td>
</tr>
<tr>
<td>H₂O₂</td>
<td>5.4</td>
<td>(48, 54)</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diquat/paraquat</td>
<td>Effect of melatonin</td>
<td></td>
</tr>
<tr>
<td>Toxins &amp; drugs</td>
<td>LD₅₀: 3.3-fold lower</td>
<td>(53)</td>
</tr>
<tr>
<td>Sulfur mustard</td>
<td>Toxicity reduction</td>
<td>(55)</td>
</tr>
<tr>
<td>Butafenacil</td>
<td>Higher antioxidant efficiency</td>
<td>(56)</td>
</tr>
<tr>
<td>Paraquat</td>
<td>Higher photosynthetic efficiency</td>
<td>(57)</td>
</tr>
</tbody>
</table>

In addition, the cascade action that is generated during the scavenging activity of melatonin is noteworthy. Thus, in its action against ROS/RNS, melatonin is transformed into a family of compounds that have been detected in vivo, and that are also capable of acting as scavengers. These products are well characterized and, some of them, have been detected in both animal and plant tissues. The most active melatonin-derivate from the point of view of their action as antioxidant are: cyclic-3-hydroxymelatonin (c3OHM), N₁-acetyl-N₂-formyl-5-methoxykynuramine (AFMK) and N₁-acetyl-5-methoxykynuramine (AMK) (47, 58). As with melatonin, these three derivatives also show high efficiency as scavengers of ·OH and, particularly, of ·OOH in the case of c3OHM. Finally, it is important to note that there are data indicating that melatonin appears to act as a more effective scavenger than vitamin E and C. Possibly being an amphipathic molecule, it can interfere with free radicals in both hydrophilic
and lipophilic environments. In fact, melatonin behaves as an excellent inhibitor of the initiation of lipid breakdown and c3OHM in the propagation (29).

5. MELATONIN IN THE ROS/RNS NETWORK IN PLANTS

The redox network can be defined as the set of genes, enzymes, metabolites and cofactors which guarantee correct homeostasis from the point of view of the redox potential in cells and tissues. In other words, a system that ensures a balance between the potentially oxidizing power of cells and antioxidant agents. When the redox system is unbalanced towards an excess of ROS and RNS, these compounds appear to show a high degree of toxicity, whereas if the system works correctly (the balance is in equilibrium), then the “good side” of ROS/RNS becomes apparent, acting then as signals in the cell that can regulate important cellular processes (28, 31, 32, 59-61). Oxidative and nitrosative stress is the terminology used when an increase of ROS and RNS, respectively, appears in the cell or in any particular organ or cell compartment (mitochondria, chloroplast, peroxisome, nucleus, endoplasmic reticulum, plasma membrane, apoplast, etc.). Therefore, two types of stress can be referred to: eustress (beneficial stress) when there is a moderate degree of stress and distress (harmful stress), when the stress is very high, sudden and/or continuous and therefore causes serious damage. If the latter is not counteracted, it can lead to irreversible and serious oxidative and degradation effects and, finally, to cell death (21, 62, 63), as can be seen in Figure 1.

![Fig. 1. General model of relationship of stressors, ROS/RNS and melatonin.](image_url)

The level of eustress or distress toward the same factor is not always the same due to the process of adaptation of individual plants. According to the hormesis concept, a stressor is considered potentially harmful when it disrupts homeostasis, and the response can be observed as a reparative process that slightly or modestly overshoots the original homeostatic level (64, 65). In this sense, several stressors disrupt homeostasis at molecular level by inducing adaptive responses in organisms that cause increased growth and induce defense processes against biotic and abiotic stress in several crops. This allows the organism to acclimate to its new environment, a key factor in the evolutionary process. Therefore, these ROS/RNS may be potentially toxic due to their nature, and, especially, to their local concentration or particular physiological status, but will also be essential as signaling molecules in adaptative physiological processes.

Thus, melatonin can regulate ROS/RNS levels through their direct chemical interaction, mainly inactivating the radical species of oxygen and nitrogen, thus contributing to redox homeostasis (Figure 1). In this case, the action of melatonin is referred to as a receptor-independent action.
6. MELATONIN AND GENE REGULATION IN THE REDOX NETWORK

Numerous genes are up- and/or down-regulated in plants by melatonin under different conditions. Surprisingly, the number of genes regulated directly or indirectly due to exogenous melatonin in plant tissues far exceeds all the expectations suggested in the 2000-2005, when only a curious chemical similarity with auxin was established (66, 67). Melatonin affects the expression of a great diversity of gene elements in different physiological conditions such as: responses to abiotic stressors (drought, salinity, cold, heat, heavy metals and combinations of them), responses to pathogen attack (fungi, virus and bacteria), in the senescence of leaves and fruits, in parthenocarpy, during growth and rooting, among others (50, 68, 69).

The existence of a molecule that functions as a sensor or receptor for a plant hormone is essential. In mammals, melatonin acts through its interaction with two types of receptors, MT1 and MT2 (70). The melatonin-receptor interaction triggers the cascade of events, including biochemical, cellular and physiological response, in what is called the signal cascade. In plants, there have always been many doubts, including disbelief, about the existence of a melatonin receptor. It was in 2018 when the first melatonin receptor in Arabidopsis thaliana was identified, called CAND2/PMTR1 (71). This receptor, located in the plasma membrane, is able to interact with G-protein α subunits, activating RbOHs and promoting Ca$^{2+}$ and K$^{+}$ fluxes that result in stomatal closure.

With respect to the plant redox network, melatonin seems to play a decisive role in two aspects: i) as a signaling molecule to initiate a response through a specific-receptor, and ii) as a regulator molecule to control ROS/RNS in a receptor-independent manner as mentioned above.

Figure 2 shows a model that integrates the two types of melatonin actions in the redox network. Its direct action (without receptor mediation) on ROS and RNS, and its action, mediated by the receptor, which involves the induction of the expression of several genes involved in the regulation of the network. Thus, melatonin acts chemically by scavenging several ROS and RNS such as ·OH, ·NO and ONOO$^-$ (see table 4), regulating the excess of these dangerous chemicals, in an attempt to ensure that the stress does not exceed the limits that would lead to distress (see Figure 1). The endogenous levels of melatonin in the homeostatic state will be decisive in this first fast line of control. An excess of ROS/RNS levels induces an interesting response, the expression of the melatonin biosynthesis genes (TDC, T5H, SNAT, COMT and ASMT), which leads to an increase in the level of melatonin in the stressed tissues (50). A common response in all types of stress studied is the endogenous increase of melatonin, due to the induction of its biosynthesis. Either as a result of the increase in endogenous melatonin through the induction of its biosynthesis genes, or as a result of the use of exogenous melatonin, a direct ROS/RNS antioxidant action by melatonin is observed. Also, an induction in the expression of multiple genes involved in the biosynthesis and degradation of these radical species has been described. Thus, melatonin induces the expression of RbOHi that generate O$^{2-}$, and also SOD, which leads to an increase in H$$_2$$O$_2$ levels (50, 72). Melatonin also induces the expression of several enzymes responsible for the detoxification of excess H$$_2$$O$_2$ such as, catalases, peroxidases and peroxiredoxins, thus being able to control the levels of ROS and cushioning the excess (see examples in (50)). Also, elements of the ASA-GSH cycle are regulated by melatonin (73-75).
**Fig. 2. Integrative model of ROS and RNS with melatonin as main regulator.**

1. Abiotic/biotic stressors provoke the first changes in the ROS/RNS balance. 2. The increase of ROS/RNS levels generates oxidative/nitrosative stress. 3. ROS and RNS (especially $O_2^-$, $H_2O_2$ and ·NO) are capable of inducing the expression of the melatonin biosynthesis genes (TDC, T5H, SNAT, COMT and ASMT). 4. An increase in melatonin levels occurs as a result of the biosynthesis of endogenous melatonin. This response can be simulated or reinforced by exogenous melatonin. 5. Melatonin, through interaction with its receptor ($R=\text{CAND2/PMTR1}$) interaction induces the expression of several enzymes such as: 6. nitric oxide synthase (NOS-like) and nitrate reductase which increase ·NO levels, and RbOH and SOD, increasing $O_2^-$ and $H_2O_2$ levels, and of $H_2O_2$-degradative enzymes, among others. 7. As a result, ROS and RNS levels are controlled by biosynthesis and degradative enzymes and (8) also regulated by the direct action of melatonin (and its by-products) through their scavenging action. 9. The level of ·NO, regulated by its biosynthesis enzymes, by direct interaction with melatonin and by its interaction with $O_2^-$-forming ONOO-, can trigger its signaling cascade response. Blue lines indicate chemical interactions between ROS, RNS and melatonin. Red lines indicate enzymatic biosynthesis or the degradation of ROS/RNS. Brown lines indicates upstream expression of related enzymes by melatonin.

Melatonin also induces the expression of some enzymes of the ·NO biosynthesis pathway such as NR and NOS-like (35, 76-80). In this case, melatonin increases the levels of a radical species of nitrogen, which can lower the levels of $O_2^-$ by forming ONOO$^-$ and therefore ·OH and ·NO$_2$, increasing the set of ROS/RNS. Generally, in most plant stress studies, melatonin causes a burst in the levels of $H_2O_2$ and ·NO. In the model of Figure 2, some pathways of biosynthesis and catabolism of ROS/RNS are presented. Lastly, ROS/RNS levels will be regulated by the direct (chemical scavenging) or indirect (induced enzymes) action of melatonin, with the aim of controlling the excesses of ROS/RNS so as not to exceed the limits of eustress (see Figure 1).

As a final result or consequence, ·NO appears, which has been seen to mediate in practically all the actions in which melatonin is involved. In a similar way, ·NO in humans plays a key role protecting against stress situations (81). In aqueous solution, the rate of autoxidation of ·NO is concentration-dependent. In physiological conditions, the half-life of ·NO reacting with $O_2$ may be several hours, but when ·NO react with Fe$^{2+}$ in erythrocytes its half-life will...
be < 1 µs (Table 1) (37, 82). The charge neutrality of ·NO facilitates its free diffusion in aqueous solutions and across cell membranes, being its diffusion constant 1.4-fold larger than that of O₂ (37).

Table 5 shows some of the most significant studies on the relationship between ·NO and melatonin in plants. Given the complexity of the mechanism that may be operating in this redox network, it is difficult to place each element in a series of linear events. Therefore, there are even doubts concerning whether ·NO is an element that acts upstream or downstream of the action of melatonin. The most recent data indicate that the activation of the ·NO signaling cascade is a consequence of the action of melatonin. In fact, many of the functions or roles attributed to melatonin were earlier attributed to ·NO, since studies on ·NO began much earlier. Excellent reviews on the functions and changes in the genetic expression in plants by ·NO can be consulted (27, 78, 83-87). Generally, this gasotransmitter has been related to plant responses associated with abiotic stress (drought, salinity, cold, heat and heavy metals), although also in many other physiological processes. ·NO is an excellent candidate to be a signal messenger acting at short distances (organelle and cell level), while melatonin is a perfect candidate to act as a long distance messenger transmitter due to its stability and amphiphilic nature. Melatonin can be transported easily via the xylem from roots to leaves and other organs (88, 89).

### Table 5. Some relevant studies on the ·NO and melatonin relationship in plants,

<table>
<thead>
<tr>
<th>Specie</th>
<th>Relevant results by melatonin and/or ·NO treatment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis thaliana</td>
<td>Improve innate immune response to bacteria</td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td>Improve innate immune response to bacteria/MAPK signaling</td>
<td>(90)</td>
</tr>
<tr>
<td></td>
<td>Improve root growth against aluminum toxicity</td>
<td>(35)</td>
</tr>
<tr>
<td>Brassica napus</td>
<td>Improve iron deficiency tolerance</td>
<td>(91)</td>
</tr>
<tr>
<td>Capsicum annuum</td>
<td>Improve tolerance to salinity stress</td>
<td>(92)</td>
</tr>
<tr>
<td>Helianthus annuum</td>
<td>Improve tolerance to iron deficiency and/or salinity stress</td>
<td>(93)</td>
</tr>
<tr>
<td></td>
<td>Glutathione metabolism regulation in salinity</td>
<td>(94)</td>
</tr>
<tr>
<td></td>
<td>Improve salt stress by SODs differential expression</td>
<td>(95)</td>
</tr>
<tr>
<td>Medicago sativa</td>
<td>Improve drought stress response through changes in proline metabolism</td>
<td>(96)</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>TDC and COMT regulation in Cd stress</td>
<td>(97)</td>
</tr>
<tr>
<td>Pyrus communis</td>
<td>Delay of postharvest senescence by ethylene synthesis inhibition</td>
<td>(98)</td>
</tr>
<tr>
<td>Solanum lycopersicum</td>
<td>Adventitious root formation</td>
<td>(78)</td>
</tr>
<tr>
<td>Solanum and Capsicum</td>
<td>Improve plant resistance to virus infection</td>
<td>(80)</td>
</tr>
<tr>
<td></td>
<td>Improve chilling tolerance in fruits by promoting polyamines and proline</td>
<td>(99)</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Fruit ripening: delays by melatonin and ·NO</td>
<td>(100)</td>
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<tr>
<td></td>
<td>Improve tolerance to lead toxicity</td>
<td>(102)</td>
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</table>
·NO is also involved in most actions mediated by phytohormones. Thus, there are several proposed mechanisms in which ·NO acts upstream of plant hormone signals, generally in the action of ethylene, jasmonic acid, salicylic acid, but also of auxin and abscisic acid. Some of these models can be consulted in excellent papers and reviews (30, 86, 98, 103-107).

Some data exist on the cascade of signal transduction used by ·NO, which includes three pathways: i) a MAPK dependent pathway through which ·NO induces the expression of MAPKs, and some Na⁺-H⁺ antiporters (30, 108); ii) a Ca²⁺ dependent pathway, where calmodulin and related proteins mediate in Ca²⁺ release through plasma membrane channels in response to several abiotic stresses. ·NO acts upstream in the Ca²⁺-calmodulin system in stress tolerance responses; and iii) a G-protein dependent pathway has been described in maize under salinity stress (109). G-protein signaling mediates the generation of ·NO and the expression of antioxidant enzymes and RbOH are up-regulated (30). This pathway is very closed to that described recently for the melatonin receptor CAND2/PRMT1 (71). In all three types of cascade signal pathways, the participation of melatonin has been described (69, 104, 110). Logically, the fact that the phytomelatonin receptor has been identified adds weight to the idea of melatonin acting as a messenger of signals, leaving for the study and controversy the possible category of plant hormone. At this point, we must not overlook the importance of ROS/RNS, especially ·NO and GSNOs, in the post-translational modifications of protein residues such as tyrosine nitration, metal nitrosylation and S-nitrosylation which modulate the activity and function of target proteins and make up an important part of the epigenetic regulation (30, 111, 112). Also, in this regard, the increasing attention given to hydrogen sulfide (H₂S), another plant messenger signaling gas, must be remembered (111, 113-117).

In summary, as already suggested in 1995 by Prof. Barry Halliwell: everything is a matter of balance (118). We propose that the balance between ROS and RNS, and the balance between these chemical species and melatonin, will determine the equilibrium or homeostasis of the redox network. Everything seems to indicate that melatonin plays a relevant role in the control - both direct (as scavenger) and indirect (as gene regulator) - in the levels and flux of the species (ROS/RNS), which will act as messengers in many cellular and physiological responses.

Regarding future perspectives, the chemical interactions between melatonin, ROS/RNS and other antioxidants under different conditions should be studied. One objective to study could be whether there are several types of receptors for melatonin in plants, and how the melatonin signal transduction chain works and by what and how many other inductors it is shared. Or is it only through the signal transduction chain of ·NO that melatonin operates? How do physiological responses differ, depending on the type of stress, and if all of them operate through melatonin and ·NO? Many molecular aspects in which melatonin is involved remain to be revealed. Another aspect of agronomic interest is the study of the effectiveness and possible toxicity or resilience of the application of synthetic melatonin in crops. Although much has been learnt in a relatively short time, it is clear that this voyage of discovery has only just begun.

ACKNOWLEDGEMENT

No financial support available for this review.

AUTHORSHIP

The manuscript was conceived by M.B. Arnao and written by M.B.A. and J.H-R.
CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

ASMT: acetylserotonin methyltransferase; AFMK: N1-acetyl-N2-formyl-5-methoxykynuramine; AMK: N1-acetyl-5-methoxykynuramine; APX: ascorbate peroxidase; ASA: ascorbic acid; ASO: ascorbate oxidase; c3OHM: cyclic-3-hydroxymelatonin; CAND2/PMTR1: melatonin receptor in Arabidopsis; CAT: catalase; COMT: caffeoyl-O-methyl transferase; Cys: cysteine; DAO: diamine oxidase; ETC: electron transport chain; GOX: glycolate oxidase; GPX: glutathione peroxidase; GSH: glutathione; GSO: glyoxysomal succinate oxidase; GST: glutathione S-transferase; LDL: low density lipoprotein; LPO: lipoxygenase; MAPK, mitogen-activated protein kinase; MT1: animal melatonin receptor type 1; MT2: animal melatonin receptor type 2; NR: nitrate reductase; NiR: nitrite reductase; Ni-NOR: plasma membrane-bound protein nitrite:nitric oxide reductase; NOS-like enzyme: plant nitric oxide synthase; OXO: oxoglutaldehyde oxidase; PAO: polyamine oxidase; PER: peroxidase; PRX: peroxiredoxin; PSI: photosystem I; PSII: photosystem II; PUFA: polyunsaturated fatty acid; RbOH: respiratory burst oxidase homolog; ROS: reactive oxygen species; RNS: reactive nitrogen species; SNAT: serotonin N-acetyl transferase; SOD: superoxide dismutase; T5H: tryptamine 5-hydroxylase; TDC: tryptophan decarboxylase; XAO: xanthin oxidase; XOR: xanthin oxidoreductase.

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