**Title:**

Melatonin’s antioxidant properties protect plants under salt stress

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**Abstract:**

This article comments on: “Melatonin improves rice salinity stress tolerance by NADPH oxidase-dependent control of the plasma membrane K+ transporters and K+ homeostasis”.

**Text:**

**Redox signaling**

Some reactive compounds appear spontaneously inside cells and apoplasts that tend to snatch electrons from surrounding molecules. Those oxidizing agents come in a variety of toxic species that altogether form so-called reactive oxygen species (ROS). The saturation of electron transfer reactions from respiration or photosynthesis as well as several environmental conditions such as salt stress, drought or heavy metals induce the formation of ROS. Biochemical reactions, such as the reduction by glutathione and ascorbate, are vital for life with aerobic metabolism by buffering redox potentials and giving cells the ability to cope with toxic ROS concentrations (Taverne, Merkus, Bogers, Halliwell, Duncker *et al.* 2018). However, ROS are not merely a stress byproduct, they play a critical role in signal transduction pathways during development, biotic and abiotic stress and adaptive responses regulating stomata aperture and pollen tube growth (Mittler, 2017; Waszczak, Carmody & Kangasjarvi, 2018, Wudick & Feijo, 2014). Respiratory burst NADPH oxidases (*RBOH*) on the plasma membrane are key components of the redox-dependent signaling cascade. The induction of these enzymes in response to stimuli sustains the fast and transient formation of hydrogen peroxide (H2O2) in the apoplast; largely unknown downstream events trigger a cellular adaptive response.

The manuscript by Juan Liu and colleagues addresses the role of melatonin during salt stress responses (Liu, Shabala, Zhang, Ma, Chen *et al.* 2020). Melatonin (N-acetyl-5-methoxy tryptamine) is a tryptophan-derived indolamine, from the same chemical family as auxin. In mammals, melatonin is best known as a hormone secreted in the pineal gland with a major role in resetting the circadian clock (Khan, Numan, Khan, Lee, Imran *et al.* 2020). It has also been characterized as one of the most potent antioxidant molecules with putative roles in aging (Reiter, Tan, Poeggeler, Menendez-Pelaez *et al.* 1994). Melatonin has been found in prokaryotes and plants where it acts as a growth factor and participates in adaptation to stresses, notably rescuing plant growth when subjected to salt stress (Khan *et al.*, 2020, Zhan, Nie, Zhang, Li, Wang *et al.* 2019). Liu *et al.* now challenge the hypothesis that melatonin’s direct involvement in the salt stress response is through redox signaling. ROS are diverse in nature, chemical reactivity and biological activity (Richards, Wilkins, Swarbreck, Anderson, Habib *et al.* 2015). While H2O2 is the best characterized ROS, many other types can form in tissues. Arabidopsis plants under salt stress experience an increase of H2O2 and hydroxyl radicals (•OH) among other ROS (Richards *et al.*, 2015). The work by Liu and colleagues tackles the question of ROS diversity and their differential biological effect.

**Melatonin reduces K+ leak from the root**

A strategy to cope with salt stress employed by plants is to keep a high [K+]/[Na+] ratio inside the cell (Flowers & Läuchli, 1983). Na+ is a chaotropic agent and its accumulation in cells has detrimental effects on enzymatic activities. A high NaCl concentration in the soil is known to induce a K+ efflux that further decreases the [K+]/[Na+] ratio. The use of non-invasive ion-selective vibrating probe (referred as MIFE in the manuscript) allowed Liu *et al.* to demonstrate that melatonin limits the detrimental K+ efflux induced by high NaCl concentrations in roots from rice. They also show that melatonin limits a K+ efflux induced by •OH. Interestingly, melatonin had no effect on H2O2-induced K+ effluxes. To further detail the interaction between ROS and melatonin, the authors focus on H2O2. They present convincing genetic data that RBOH activity is required for the inhibitory effect of melatonin on NaCl -induced K+ effluxes. They propose an elegant model to reconcile two apparently contradictory results: on one hand, melatonin does not rescue H2O2-dependent K+ efflux, while melatonin needs H2O2 production by RBOH enzymes to limit K+ effluxes during salt stress. The model proposed by the authors is that melatonin has a ROS scavenger activity with specificity to eliminate •OH over H2O2. •OH are more toxic than H2O2 and the specific scavenging would allow a distinct H2O2-dependent signaling pathway. If experimentally confirmed, this mechanism could prove significant for the other physiological functions involving ROS signaling. A transcriptomic analysis of the melatonin effect in Arabidopsis root revealed a large number of genes affected (>400). The amount of transcriptomic variations make it difficult to deduce strong conclusions regarding the molecular mechanism dependent on melatonin, but do indicate a transcriptional upregulation of stress-response genes, notably K+ transporters and channels.

**Melatonin acts early in response of root cells to salt stress**

We made the exercise of introducing melatonin into a scheme starring the main known players of the root response to salt stress in glycophytes (Fig.1). (i) Salt stress starts by Na+ entry into the cell through non-selective cation channels. Na+ extruding transporters at the plasma membrane (PM) or tonoplast eventually get overloaded. As cytosolic accumulation of Na+ increases, the [K+]/[Na+] ratio decreases. (ii) Na+ influx depolarizes the PM triggering a K+ efflux through non-selective cation channels and outward K+ channels, further decreasing the [K+]/[Na+] ratio. The adaptation to NaCl implies the activation of ion transporters that re-establish a healthy [K+]/[Na+] ratio (Wu, Zhang, Giraldo & Shabala, 2018). The ion transport system that maintains a high [K+]/[Na+] ratio is under the control of a signaling network involving H2O2 and Ca2+ that can be schematized as follows; (a) Na+ is sensed, possibly by the newly described Na+ sensor glucuronosyltransferase for glycosyl inositol phosphorylceramide (GIPC) sphingolipids (Jiang, Zhou, Tao, Yuan, Liu *et al.* 2019). (b) Following Na+ sensing, Ca2+ channels are activated and (c) Ca2+ activates RBOH by direct interaction with the enzyme. (d) H2O2 induces a transcriptional regulation of K+ transporters and signaling proteins. In parallel, H2O2 and Ca2+ also directly target ion transporters for post-translational regulation. (e) The transporters newly transcribed or targeted by H2O2/Ca2+ regulation contribute to maintain high [K+]/[Na+] ratio.

Other ROS proliferate during salt stress, including •OH through the Fenton or Haber-Weiss reactions using H202 that has either freely diffused across the PM or generated from the mitochondria. Non-H2O2radicals, like •OH, are highly toxic by their damaging effect on membranes. According to Liu *et al*. melatonin favors the H2O2 signaling pathway by its antioxidant property of scavenging •OH. The model implies that melatonin scavenges non-H2O2 ROS, preventing the interference of H2O2-dependent signaling pathway.

**An emerging understanding of ROS signaling eclipsed by a complex biochemistry**

What we referred to here as redox signaling implies homeostatic mechanisms for ROS as well as controlled bursts of ROS synthesis. ROSs further oxide unidentified targets that activate transduction pathway. One can measure the redox potential of *one* given *reaction* to monitor associated signaling events. The redox potential of a reaction is the equivalent to pKa for reactions involving proton exchanges, but instead of characterizing the free energy of H+ transfers, it characterizes electron transfers. The solvent molecule H2O couples H+ exchange reactions together, and the pH characterizes the general H+ availability in a solution. pH or pCa can be measured that quantifying the proton and calcium availability in the solution in order to monitor respective H+ and Ca2+ signaling. There is no general coupler of redox reactions happening in a biological system, and only redox potentials for *individual reactions* can be measured, and not a “cell redox potential”. Redox signaling rather involves a network of interacting chemical reactions that have to be characterized individually.

By its biochemical nature, redox signaling is therefore complex and the work by Lui *et al.* tackles this complexity, introducing competition between two ROS (•OH and H2O2) and two physiological effects (toxicity versus signaling of ROS). The biology of redox signaling remains a whole continent to discover. Understanding the physiology of redox signaling and underlying molecular mechanisms is a challenging aspect in modern physiology, and will imply detailing the different players, their sensors, targets and interactions. H2O2 and •OH are not the only ROS molecules, they interact with other agents including the nitrogen reactive species such as nitric oxide (NO) signaling molecule. H2O2 and other ROS also interact with other redox reactions essential for plants, possibly 1-aminocyclopropane carboxylic acid (ACC) oxidation to ethylene for instance, a hormone precisely involved in oxidative stress response.

**Figure 1: ROS induced damage and signaling under salinity (NaCl) stress based on melatonin.**

Possible molecular mechanism of melatonin that confers salt stress resistance (see text for details). What accounts for differential salt tolerance between glycophytes and halophytes is the ability to maintain high [K+]/[Na+] ratio. Note that in addition to the Na+ -specific stress addressed here, salt stress has a Cl- component that was omitted for simplicity. Players starred: **(i)** Channels permeable to Na+ of unknown nature let Na+ permeate inside the cell. Glutamate receptor-like channels, cyclic-nucleotide gated channels or annexins could be involved. **(ii)** Na+ influx depolarizes the membrane, K+ leaks out of the cell through unidentified channels, possibly non-selective channels from (i) as well as depolarization-activated channels such as SKOR. **(a)** Extracellular Na+ is sensed, possibly by GIPC sphingolipids. **(b)** Unidentified channels permeable to Ca2+, possibly by transporters similar to those listed for (i) induce a Ca2+ signal and trigger so-called SOS pathway. **(c)** RBOH activity is activated by Ca2+ interacting with EF: EF-hands. The Fenton reaction (Fe2+ + H202 🡪 Fe3+ + •OH + OH-) and Haber-Weiss Reaction (O2•- + H202 🡪 O2 +•OH + OH-) participate in •OH formation that is scavenged in the presence of melatonin. **(d)** H2O2 induces transcriptional regulation through an unknown pathway. **(e)** Transporter regulation helps maintain high [K+]/[Na+] ratio. Possible transporters recruited include: PM H+-ATPases and tonoplast H+-ATPases/H+-PPases as well as K+ transporters such as AKT, HAK, SKOR (known to be regulated by redox), Na+/H+ exchangers (NHX) and the antiporter Salt Overly Sensitive 1 (SOS1). Those transporters may also be the direct target of ROS by direct oxidation of cysteines.

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**References**

Flowers T.J. & Läuchli A. (1983) Sodium versus potassium: substitution and compartmentation. *Inorganic Plant Nutrition*, **15b**, 651–681.

Jiang Z., Zhou X., Tao M., Yuan F., Liu L., Wu F., Wu X., Xiang Y., Niu Y., Liu F., Li C., Ye R., Byeon B., Xue Y., Zhao H., Wang H.N., Crawford B.M., Johnson D.M., Hu C., Pei C., Zhou W., Swift G.B., Zhang H., Vo-Dinh T., Hu Z., Siedow J.N. & Pei Z.M. (2019) Plant cell-surface GIPC sphingolipids sense salt to trigger Ca(2+) influx. *Nature*, **572**, 341-346.

Khan A., Numan M., Khan A.L., Lee I.J., Imran M., Asaf S. & Al-Harrasi A. (2020) Melatonin: Awakening the Defense Mechanisms during Plant Oxidative Stress. *Plants (Basel)*, **9**.

Liu J., Shabala S., Zhang J., Ma G., Chen D., Shabala L., Zeng F., Chen Z.H., Zhou M., Venkataraman G. & Zhao Q. (2020) Melatonin improves rice salinity stress tolerance by NADPH oxidase-dependent control of the plasma membrane K+ transporters and K+ homeostasis. *Plant Cell Environ*.

Mittler R. (2017) ROS Are Good. *Trends Plant Sci*, **22**, 11-19.

Reiter R.J., Tan D.X., Poeggeler B., Menendez-Pelaez A., Chen L.D. & Saarela S. (1994) Melatonin as a free radical scavenger: implications for aging and age-related diseases. *Ann N Y Acad Sci*, **719**, 1-12.

Richards S.L., Wilkins K.A., Swarbreck S.M., Anderson A.A., Habib N., Smith A.G., McAinsh M. & Davies J.M. (2015) The hydroxyl radical in plants: from seed to seed. *J Exp Bot*, **66**, 37-46.

Taverne Y.J., Merkus D., Bogers A.J., Halliwell B., Duncker D.J. & Lyons T.W. (2018) Reactive Oxygen Species: Radical Factors in the Evolution of Animal Life: A molecular timescale from Earth's earliest history to the rise of complex life. *Bioessays*, **40**.

Waszczak C., Carmody M. & Kangasjarvi J. (2018) Reactive Oxygen Species in Plant Signaling. *Annu Rev Plant Biol*, **69**, 209-236.

Wu H., Zhang X., Giraldo J.P. & Shabala S. (2018) It is not all about sodium: revealing tissue specificity and signalling roles of potassium in plant responses to salt stress. *Plant and Soil*, **431**, 1-17.

Wudick M.M. & Feijo J.A. (2014) At the intersection: merging Ca2+ and ROS signaling pathways in pollen. *Mol Plant*, **7**, 1595-1597.

Zhan H., Nie X., Zhang T., Li S., Wang X., Du X., Tong W. & Song W. (2019) Melatonin: A Small Molecule but Important for Salt Stress Tolerance in Plants. *Int J Mol Sci*, **20**.