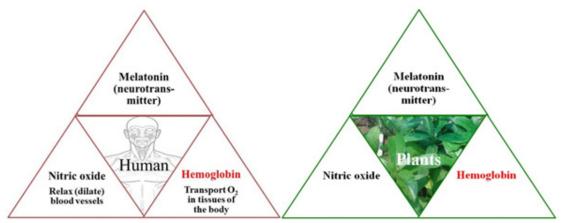
# Relieving stress? Plants and human beings engage similar molecular crosstalk Hemoglobin in plants?

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"All forms of life are blood kin under the skin and in the roots."

This statement published in *The New York Times* more than a decade ago suggests that blood is essential for all living organisms. Seems surprising. Are they also suggesting that plants too have blood? Do you think that plants have hemoglobin running in their veins? Here, we are not taking into consideration leghemoglobin, which is there in the root nodules of leguminous plants. However, non-symbiotic hemoglobin (nsHb) is, in fact, present both in monocot and dicot plants.



Melatonin cares for all – be it human beings or plants

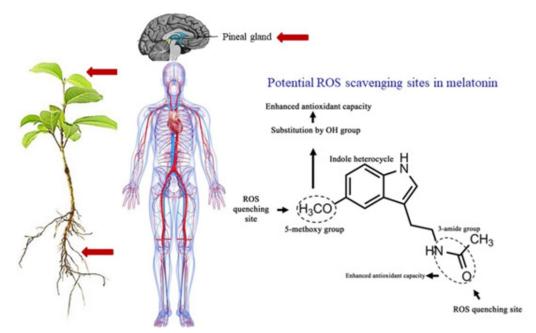
<sup>\*</sup> Dr. Neha Singh, Post Doctoral Fellow from University of Delhi, New Delhi, is pursuing her research on "Calcium and Auxin Signaling during Adventitious Root Development and Analysis of Possible Nitric Oxide Crosstalk." Her popular science story entitled "Relieving Stress? Plants and Human beings Engage Similar Molecular Crosstalk" has been selected for AWSAR Award.

Research has revealed that nsHb is involved in myriads functions in plants, including abiotic stress tolerance, hypoxia, salt and cold stress and, it acts as an excellent nitric oxide (NO) scavenger.

## Versatile roles of NO in humans and plants

NO, a well-known gaseous molecule and for whose roles in vasodilation in human beings, Nobel Prize was awarded to scientists in the year 1998, is also biosynthesized in plants. NO is exceptionally important for controlling blood pressure. In plants, NO is not only involved in regulating growth and development of plants under normal growth conditions, it also regulates many plant responses both under abiotic and biotic stress conditions. It regulates diverse functions of proteins through interaction with various metallic constituents of proteins, such as heme-iron, zinc-sulphur, iron-sulphur and copper. Thus, it modifies the protein structure/function. NO can also cause tyrosine nitration of proteins, which is considered as a significant biomarker of nitrosative stress. Tyrosine nitration can, subsequently, lead to activation or inhibition of target proteins. NO either promotes or inhibits the activity of a variety of hemoproteins.

Now we all know that, plants also have hemoglobin and NO like human beings, do they also have 'brain' where all responses are controlled by neurotransmitters? Surprisingly, melatonin, initially discovered as a neurotransmitter in humans and animals, is also present in plants. In human beings, it is produced in the pineal gland and regulates daily circadian rhythms like sleep and wakefulness. Interestingly, in plants it is produced in root and shoot tips and it acts as a potential antioxidant which scavenges free radicals or reactive oxygen species (ROS).

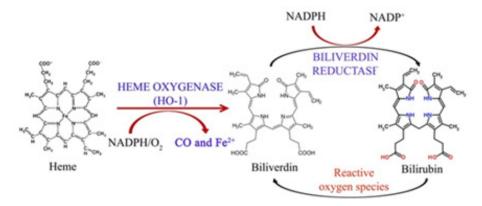


Sites of melatonin biosynthesis in plants and human beings

## Stress impacts plants and humans alike

These are the biomolecules (hemoglobin, NO and melatonin) whose independent actions or cross-talk with each other I have investigated in sunflower seedlings raised under NaCl stress. In India, salinity stress is a major factor that reduces crop productivity. Many states in India, including Andhra Pradesh, Tamil Nadu, Karnataka, Haryana, Punjab, Bihar, Odisha and Madhya Pradesh, have salt-affected agricultural land. Ever-increasing global warming is causing an increase in sea level thereby inundating coastal agricultural land masses. Sunflower is an important oil crop whose productivity is drastically reduced due to salinity stress. The present work aims to understand the physiological and biochemical mechanisms of salt stress tolerance utilizing the actions of NO, hemoglobin and melatonin.

Whenever man is under stress, a number of ROS are released in blood stream. So is also the case with plants. Plants also exhibit enzymatic or non-enzymatic antioxidant machinery to combat against prevailing stress conditions. The well-known enzymes that detoxify plant cells with harmful radicals or ROS are catalase, various peroxidases, superoxide dismutase, glutathione reductase and ascorbate peroxidases. Recently, I investigated a novel antioxidant enzyme-'heme oxygenase' (HO) in plants. Its expression and modulation was also studied in seedling cotyledons in response to salt stress. It is interesting to know the functions of HO in human beings. Many of us are aware of the fact that during jaundice, levels of bilirubin increase enormously in our body. In humans, HO is localized in liver and plays an important role in blood recycling. It is also responsible for the death of blood cells as it breaks down the heme produced via disintegration of hemoglobin from blood cells. Thus, it degrades heme molecule present in the blood to release biliverdin, which is subsequently converted into bilirubin. Both biliverdin and bilirubin exhibit antioxidant potential in human beings and also in plants. It would be interesting to know the antioxidant potential of this enzyme during early stages of sunflower seedlings growth under salt stress. Heme oxygenase, being a hemoprotein (heme containing enzyme), its function can efficiently be modulated by NO.

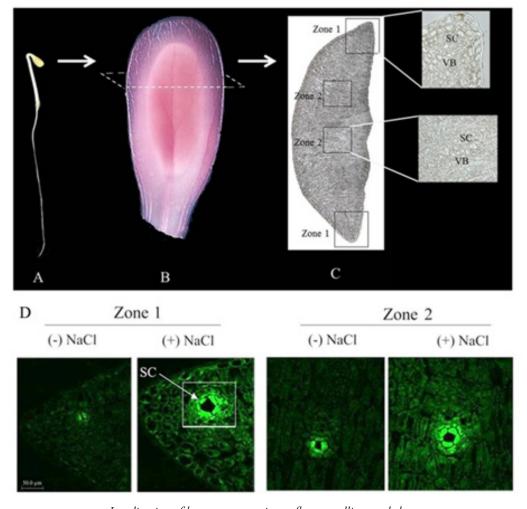


Heme oxygenase as an antioxidant enzyme in human beings and plants

In plants, HO is localized in plastids and is majorly involved in the synthesis of light signaling molecules, that is, chromophore of phytochrome. HO has three isoforms in plants. HO-2 and HO-3 are constitutively expressed. HO-1 is inducible isoform, which is induced by a variety of stress conditions such as, salt, cold, flood, UV-B radiation. Hence it is responsible for protecting plants under stress conditions.

## Differential spatial distribution of heme oxygenase holds the key

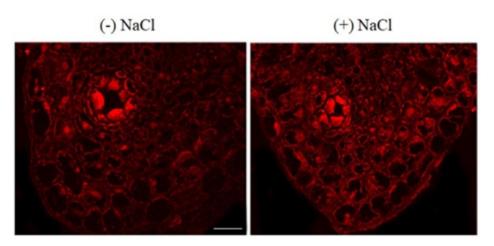
Immunolocalization of HO-1 by confocal laser scanning microscopy (CLSM) revealed very astonishing result as this enzyme was found to be abundantly present in the specialized cells surrounding the secretory canal in seedling cotyledons raised under salt stress conditions. Secretory structures, including secretory canals, constitute an important anatomical feature in many plants.



Localization of heme oxygenase in sunflower seedling cotyledons.

Therefore, secretory canals are likely to play an important role in signaling of diverse biomolecules and ions in plant cells thereby affecting long-distance sensing of stress from root to shoot. Such a modulation of spatial distribution of heme oxygenase in response to NaCl stress implicates its role in long-distance sensing of salt stress in sunflower seedling cotyledons for the transport of signaling molecules.

Enhanced accumulation of NO was also demonstrated in seedling cotyledons in response to salinity stress. Immunolocalization of tyrosine nitrated proteins in seedling cotyledons by CLSM revealed the abundance of tyrosine nitrated proteins as well in the specialized cells surrounding the SC where high expression of HO-1 was noted. Thus, NO was found to be positively modulating HO-1 activity by way of its interaction/binding with heme group, which serves both as a substrate and as a prosthetic group for HO-1.

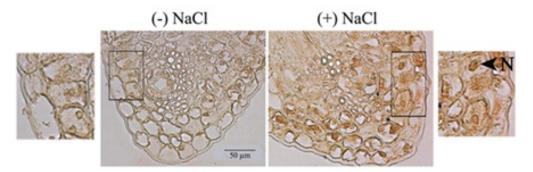


Immunolocalization of tyrosine nitrated proteins.

### Melatonin cross-talk with ROS and NO

Melatonin, a well-known neurotransmitter, was analyzed in seedling cotyledons in the absence or presence of NaCl stress. Its enhanced accumulation was observed under salinity stress. It is in congruence with increased HO-1 activity in (+) NaCl condition. It is thus evident that melatonin acts as a long-distance signaling molecule in response to salt stress and changing HO-1 activity.

Under stress conditions, NO burst is observed as a mechanisms to tolerate stress but at higher concentration it can interact with other ROS species and generate reactive nitrogen species like, dinitrogen ( $NO_2^{\square}$ , responsible for tyrosine nitration of proteins) and peroxynitrite. The main role attributed to nsHb in plants is its ability to efficiently scavenge excess NO production. In fact, it is a very interesting observation that if we provide hemoglobin exogenously in growth medium, it can scavenge most of the endogenous NO from the tissue system. Also, it can scavenge an important ROS species, that is, hydrogen peroxide. The role of exogenously supplied hemoglobin as a potent



Effect of salt stress on melatonin accumulation in the cells of seedling cotyledons.

NO scavenger was further strengthened as it was able to significantly enhance the levels of ethylene biosynthesis enzyme-ACC oxidase in its presence. However, in the presence of NO donor the activity of this enzyme is highly reduced. Thus, application of hemoglobin antagonized the action of NO on ethylene biosynthesis enzyme. Discovery and functions of hemoglobin in plants enable us to enter into a new era of research whereby we can make plants healthier and better adapted to stress conditions via enhancing the expression of genes encoding nsHb in plants.

It is thus, evident that, unlike human beings, plants cannot run away from stress conditions but they utilize similar molecular or enzymatic machinery as present in human beings to combat stress and maintain their growth and development. To build a strong foundation, application of knowledge from fundamental plant physiology or biochemical events is thus necessary to understand its application in human welfare.

And the story goes on....