***Review Paper:***

# Interaction between Nitric Oxide and Hydrogen Sulfide in Abiotic Stress Challenged Plants

**Chandrakar Vibhuti[1], Kumar Meetul[2] and Keshavkant S.[1*]**
1. School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, INDIA
2. Directorate of International Cooperation, Defense Research and Development Organization, New Delhi, INDIA
*skeshavkant@gmail.com

## Abstract

*Nitric oxide (NO) and hydrogen sulfide ($H_2S$) are two versatile gaseous molecules which play myriad roles in the growth and development of plants. They play an important role in signal transduction process in plants exposed to various environmental stresses. Signal transduction and various antioxidant strategies are vital for the management of abiotic stress imposed alterations in plants. These two secondary messengers neutralize the cell perturbations caused by stress-triggered over produced reactive oxygen species. Study of crosstalk between NO and $H_2S$ reveals the functional importance of proteins regulated during S-nitrosylation and S-sulfhydration respectively, the two major signal-dependent post-translational protein modifications.*

*Also, NO and $H_2S$ decrease the toxic impacts of reactive species by triggering the signal transduction process, enhancing antioxidant enzymes, stimulating other signaling molecules and regulating the transcript levels of different stress-responsive genes. This review mainly emphasizes on the roles of NO and $H_2S$ in responses of plants to abiotic stresses and reveals the crosstalk involving NO and $H_2S$ in stress tolerance mechanisms.*

**Keywords:** Hydrogen Sulfide, Nitric Oxide, Oxidative stress, Reactive oxygen species, Signal transduction, Signaling molecules, Stress tolerance.

## Introduction

Abiotic stresses such as heat, cold, salinity, drought, metal/ loid, ultraviolet (UV) radiation etc. adversely affect the rate of germination, development and yield of economically essential crop plants and more than 50% yield losses are direct result of these stresses[23,26]. One of the most common phenomena taking place during the plant responses to these abiotic stresses is the oxidative explosion illustrated by the uncontrolled production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide ($H_2O_2$) and superoxide ($O_2^{\bullet -}$)[41].

These elevated levels of ROS are severely injurious to plant cells as they directly oxidize the lipids, proteins and amino acids, inactivate enzymes and damage pigments and nucleic acids[6,17]. Condition of oxidative stress triggers a series of detrimental impacts in plants including reduced germination score, biomass, root and shoot length, reduction in the number of leaves and leaf area, curling, wilting and necrosis of leaf blades, disturbed cellular osmotic balance, alteration in flow of energy, interference with minerals and ions uptake, losses in the mineral contents, inhibition in the rate of photosynthesis, chlorophyll biosynthesis, enzyme activities and cellular metabolism[4,37]. These damaging effects of oxidative stress hamper / hinder the growth and development and ultimately lead to death of plants.

The effectual control and rapid removal of ROS is essential for the proper functioning and survival of the plants. Thus, to counterbalance the environmental stresses, plants store multiple groups of compatible solutes such as proline, glycinebetaine, sugars etc. together with defensive enzymes and non-enzymatic components[36]. Enzymatic components include superoxide dismutase (SOD), catalase (CAT), peroxidases such as ascorbate peroxidase (APX), guaiacol peroxidase (POD) and glutathione peroxidase whereas flavonoids, glutathione (GSH), ascorbate (AsA) and α-tocopherol constitute non-enzymatic components which protect the plants against ROS-induced oxidative damage[28,35]. Thus, understanding the mode of action of some of the molecules applied exogenously that can improve the defensive system of plants, could help in the mitigation of detrimental effects of abiotic stress-induced oxidative burst.

The two important gaseous molecules viz. nitric oxide (NO) and hydrogen sulfide ($H_2S$) have crucial roles in several developmental processes of the plants and are also involved in their protection against various abiotic stresses[29]. In plants, both of these molecules are key signal messengers involved in various developmental processes such as seed germination and root organogenesis. Also, these signaling molecules elicit the antioxidant defensive mechanisms of plants to reciprocate the oxidative damage to cellular structures[33]. The interactions of NO and $H_2S$ have also been used in awarding plant tolerance to various stresses such as aluminum, arsenic (As), cadmium (Cd), salinity and heat in plants[11,16,23,32,33].

Being a ubiquitous, gaseous bioactive molecule and a secondary messenger, NO has gained an increasing attention of scientific research in plant cells. It is well known that NO has significant role in the management of plant growth, development, interaction with other signaling molecules and in the adaptive responses to the abiotic stresses[15]. In addition, its role is evident in seed germination, root formation and elongation, fruit yield, photomorphogenesis, nutrient uptake and transport, photosynthetic rate, programmed cell death, stomatal conductance and transpiration[7]. Also, NO plays a central role in the activation of some of the important enzymes including α-amylase, protease and antioxidant enzymes that contribute in the enhanced germination under stressful conditions[17].

In plants, NO can act through cyclic guanosine monophosphate (cGMP)-dependent or -independent pathway via increasing the cytosolic concentration of $Ca^{2+}$ and altering the activities of protein kinases[25]. Guanylate cyclase catalyzes biosynthesis of cGMP, a secondary messenger and a strong candidate in the regulation of cell metabolism. This soluble enzyme is the main intracellular receptor of NO. Most of the consequences of NO are recognized via accumulation of cGMP and/ or stimulation of soluble guanylate cyclase[1,25].

The protective role of NO relates to its antioxidant ability to directly quench the ROS and activation of antioxidant enzymes thereby restoring the redox balance of the cell and lessen the oxidative damage[31]. One proposed mechanism by which plants defend themselves against the harmful impacts of abiotic stresses is through the accumulations of endogenous NO. Exogenous treatment of NO alleviated the toxic effects of variety of abiotic stresses such as salinity[18], As[17], extreme temperatures[15], chromium[20], Cd[42] etc. It has been intensively studied and many reviews[14,25,31] have been published in this area. Because of having the capacity of diverse regulatory roles involved in plant metabolism and activation of adaptive stress responses, it is considered to be a 'jack-of-all-trades' molecule.

Another important signaling molecule $H_2S$ is a crucial part of the regulatory mechanism of many physiological processes in animal and plant systems[11]. In the last decade, $H_2S$ has gained much attention by the researchers in exploring its *in vivo* roles in plant development and stress management[21,26]. It has been well reported that the plant can actively generate $H_2S$ that is able to provide resistance and/or tolerance to various environmental stresses[22]. Also, increasing evidence illustrates that $H_2S$ is involved in providing protection when applied exogenously to plants grown under several abiotic stresses such as salt[34], cold[12], high temperature[26], drought[24], heavy metal[27] etc. More interestingly, sodium hydrosulfide (NaHS) and morpholin-4-ium-4-methoxyphenyl (morpholino) phosphinodithioate are mostly applied as $H_2S$ donors which showed significant positive impacts on several important phenomena including seed germination, organogenesis, photosynthesis, stomatal apertures, growth and senescence regulation[22].

Exogenous application of $H_2S$ mitigates the injuries caused due to abiotic stress-induced oxidative damage including electrolyte leakage, lipid peroxidation, protein oxidation, enzyme inactivation, etc.[10] Also, $H_2S$ leads to intensification of important cellular macromolecules and antioxidative system of plants exposed to various abiotic stresses[32]. Application of exogenous $H_2S$ also led to the accumulation of non-enzymatic antioxidants such as amino acid and carbohydrates under stressed conditions[12].

Recently, increasing studies pertaining to the defensive roles of NO and $H_2S$ in plant system indicated that these two molecules are secondary messengers in response to stress and trigger downstream signal transduction[32]. These are considered to be important signaling molecules which are capable to provide resistance to different stresses in plants. Although, the crosstalks of NO and $H_2S$ in response to plant stress still unraveled completely and necessary to be further studied. Keeping in view the various important physiological roles of NO and $H_2S$ in plants and the necessary space constraints, we confine our coverage to their involvement in signaling and their impacts in the plants exposed to some of the popularly noted stresses such as Cd, As, salinity, temperature, drought and UV-B radiation.

## Abiotic stress-induced toxicity: An overview

Exposure of various environmental stresses triggers over generation of ROS during various metabolic processes in plants which finally elicit oxidative stress condition[35]. The cell compartments such as mitochondria, chloroplasts and peroxisomes carrying out the highly oxidizing metabolic activity are the starting places for the production of ROS in plant cells[2]. Depending on the site of generation, ROS induces specific signaling pathways. These ROS are highly reactive and destructive for cellular structures and macromolecules like protein, enzyme, lipid and DNA[5].

In addition to ROS, accumulation of another cytotoxic compound, methylglyoxal (MG) content also elevated under the exposure of heavy metals and salt in plants due to perturbations in the glyoxalase and antioxidative defense systems, which leads to oxidative stress by dropping the GSH content[39].Typically, ROS arises by the relocation of one, two or three electrons, in $O_2$ to form $O_2^{\cdot -}$, $H_2O_2$ and $^{\cdot}OH$, respectively[13].

Among these, $^{\cdot}OH$ is the most toxic ROS to cellular organelles because there is no any other antioxidant enzyme for its direct elimination from the cells. The two reactions viz. Fenton and Haber-Weiss also take part in the production of toxic ROS. Abiotic stresses imbalance the generation and scavenging of ROS and MG which increases their accumulation resulting in an eventual promotion of oxidative damage[3].

Abiotic stress-induced lipid peroxidation may also occur due to higher lipolytic activity of lipoxygenase enzyme. Membrane damage due to lipid peroxidation involves a number of mechanisms such as inactivation of important membrane protein such as $H^+$ATPase, oxidation and binding with protein thiols, oxidation of amino acid residues in proteins, activation of proteases and endonucleases, inhibition in the function of the photosynthetic apparatus, alteration in the composition and fluidity of membrane lipids etc. The collective effect can lead to death of the cells[13]. An elevated level of lipid peroxidation has been revealed in many abiotic stress studies such as heat, drought, salt and heavy metals[23,24,30,33,41]. The aldehydic products of lipid peroxidation reaction can easily penetrate the membranes and cause cellular injuries by reacting with other lipids, proteins, DNA and RNA far from their site of origin[3].

In addition to aldehydic products, the oxidation of DNA takes place when •OH reacts with purine and pyrimidine bases and deoxyribose backbone, which trigger removal of nucleotides, deoxyribose oxidation, reduction in the efficiency of DNA synthesis, DNA-protein crosslinks, genomic instability and strand breakage[3,13]. This can lead to DNA mutations and inactivation of the encoded proteins. The protein entity of the cell gets covalently oxidized by ROS[40]. Under abiotic stresses, the widely used marker of protein oxidation determination is protein carbonylation that may take place due to the direct oxidation of the amino acid side chains[5].

Normally, presence of antioxidant defense system at the ROS production site helps in the mitigation of toxicity but when this system fails to cope-up with ROS generation and accumulation, there is leakage in cellular compartments[6,30]. Instantaneous production of $H_2O_2$ after heavy metal stress indicates its role in triggering signal transduction and heavy metal tolerance in plants[13]. Therefore, it is evident that the oxidative stress greatly inhibits the growth and development by perturbing the physiology and metabolism of plants[37].

To prevent from excessive accumulation of heavy metals and for their detoxification, plants possess a variety of strategies which include heat shock proteins, metallothioneins and GSH. Also, there is antioxidant protection system in plants that helps to protect them from the abiotic stress-elicited oxidative injury[30]. This system involves enzymatic and non-enzymatic low-molecular weight components. Antioxidant enzymes include SOD, POD, APX, CAT and glutathione reducatse (GR) while low-molecular weight antioxidants are AsA, GSH, tocopherol, carotenoids, quinines and some polyphenols[28].

**Effects of interaction of NO and $H_2S$:** Functional parallelism in achieving the resistance against abiotic stresses including cross-adaptation involves varied signal association that consists of many second messengers such as abscisic acid, $H_2S$, $H_2O_2$ and NO as well as their crosstalk[22]. Among these, NO and $H_2S$ share very crucial roles in the signaling cascade and promote plant adaptation to several abiotic stresses through post-translational modifications of proteins, regulatory roles in gene expression and crosstalk with the other plant regulators[29] (Fig. 1).

Recently, the degree of the cooperative functions of these two signaling messengers has attained considerable attention by the researchers[23,32,33]. In brief, $H_2S$ is generated by the action of cytosolic L-cysteine desulfhydrase that also mediates the synthesis of NO involving nitrate reductase[9]. The $H_2S$ modulates the signal cascade by following mechanisms: 1) inducing the NO mediated synthesis of 8-nitro-cGMP/8-mercapto-cGMP that elicit stomatal shutting, 2) trigger $H_2O_2$ production through phosphatidic acid and NADPH oxidase and 3) regulating the $K^+$ channels of guard cells[9]. Also, in guard cells, NO regulates the stomatal movement by modulating the intracellular concentration of $Ca^{2+}$ and cGMP synthesis[1].

According to Shi et al[32] and da-Silva et al[11], interaction of NO and $H_2S$ induces gene expression to increase the activity of $H^+$ATPase in the cell membrane and also to balance the ratio of $K^+/Na^+$ under salt stress. This signal transmission leads to the activation of the $Na^+/H^+$ antiporter enzyme that compartmentalizes and eliminates the cytoplasmic $Na^+$ ions[8]. In this way, NO trigger $H_2S$ in maintaining the redox homeostasis of the cells.

Findings of Wang et al[34] revealed that in the presence of $H_2S$ donor in salt-stressed *Medicago sativa*, level of endogenous NO increased which led to the high $K^+/Na^+$ ratio, elevated actions of antioxidant enzymes and the accumulation of their transcripts.

Similarly, in salt-stressed *Hordeum vulgare*, treatment of $H_2S$ donor increased the NO content and up-regulated the expression of genes related to $K^+$ ions channel with subsequent increment in the salt tolerance of the seedlings[8]. Exogenous NO and $H_2S$ offset the negative effects on coleoptile expansion and radicular system development elicited by exposure of heavy metal[20]. Also, NO and $H_2S$ can improve the uptake of Fe in *Fragaria ananassa* by extenuating leaf chlorosis caused due to Fe insufficiency[18].

Plant hemoglobin serves as a combined sink for NO and $H_2S$, however its binding resembles for NO as compared to $H_2S$. Because NO and $H_2S$ can react with each other, modulate similar molecular targets and cross regulate enzymatic sources. For the counterbalance, they antagonize each other despite the diversity in molecular targets[16]. To achieve the physiological response, NO and $H_2S$ mediated the signal transduction through the pathways such as cGMP signal cascade and mitogen-activated protein kinase pathways. Mainly, the cysteine residues of target proteins are modulated by NO and $H_2S$ through protein S-nitrosylation and S-sulfhydration respectively.

In protein S-nitrosation/ nitrosylation, NO is covalently added to the sulfhydryl side chain of cysteine of peptides and proteins and form protein S-nitrosothiols[25]. The stability of this covalent alteration relies on the existence of reducing agents (such as thiols and ascorbate) and trace metal ions (such as copper and iron). S-sulfhydration is the binding of $H_2S$ to metal centers of several proteins such as cytochrome-c oxidase and hemoglobin and is responsible for the oxidative alteration of cysteine to the subsequent persulfide type[9].

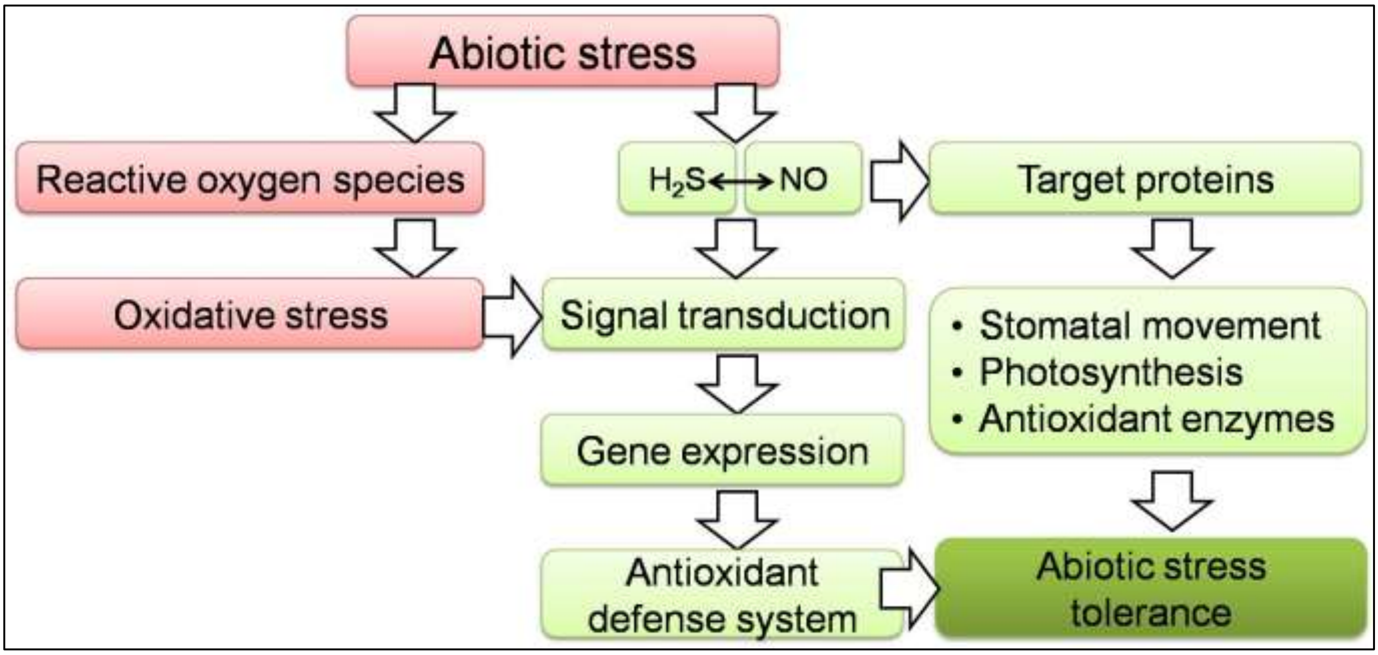


**Figure 1: Crosstalk events associated with nitric oxide (NO) and hydrogen sulphide ($H_2S$) in abiotic stress tolerance**

In addition, in the process of sulfhydration, the target cysteine residue is also S-nitrosylated[16]. The sulfhydryl group of cysteine residues of proteins is the redox switch that regulates the susceptible enzymes and efficient biological sensors of oxidative status of the cell[9]. The cysteine residues of antioxidant enzymes APX (located in cytosol, mitochondria, chloroplasts and peroxisomes) and CAT (localized in peroxisomes) have been shown to be targeted by S-nitrosylation and S-sulfhydration. This leads to finely regulated maintenance in the activities of APX and CAT mediated by NO and $H_2S$ as signal molecules and balances the equilibrium between ROS ($H_2O_2$), NO and $H_2S$. This equilibrium sometimes interrupted in response to severe stress and caused cellular distortions.[9]

**NO and $H_2S$ in salt stress tolerance:** Salt stress can reduce/ inhibit germination, growth and development of plants by affecting diverse physiological processes such as osmotic pressure, ion homeostasis and nutritional uptake[38]. High salinity can also cause oxidative stress due to the excessive ROS generation resulting in oxidative injury to cellular lipids, enzymes, proteins and DNA[30].

Wang et al[34] reported that treating *Medicago sativa* seeds with $H_2S$ under salt stress might elicit the production of endogenous NO which causes adaptation to salt stress in the growing seedlings. This increment in the level of endogenous NO further increases with the enhancement in the dose of $H_2S$ and was accompanied with the decreased lipid peroxidation reaction with simultaneous increment in the transcript levels of SOD, POD, CAT and APX genes.

Moreover, NaHS (donor of $H_2S$) decrease the efflux of $K^+$ ions with increase in $Na^+$ ions in the vacuoles, thus helping in maintaining the ionic homeostasis. Transcriptional studies by Chen et al[8] clearly revealed that under salt stress, treatment of $H_2S$ up-regulated the expression of HvAKT1 gene, a $K^+$ channel and a high-similarity protein system for its uptake (HvHAK4) in *Horduem vulgare*. Also, there was an up-regulation in the transcript levels of vacuolar $Na^+/H^+$ antiporter (HvVNHX2) and $H^+$-ATPase subunit and protein expression of vacuolar $Na^+/H^+$ antiporter[8]. These results clearly exemplified that $H_2S$ is directly involved in triggering signals for providing tolerance to varied abiotic stresses in plants.

Another line of evidence showed that treating the seedlings of *Triticum aestivum* with a NO donor increased the contents of L-cysteine and $H_2S$ in response to osmotic stress[19]. This increment in the level of $H_2S$ triggered by exogenously applied NO increased the activities of antioxidant enzymes and the contents of non-enzymatic antioxidants (glycinebetaine and proline) thus controlled the oxidative injury in osmotic stressed *Triticum aestivum* seedlings[19]. Additionally, da-Silva and Modolo[10] reported that endogenous NO and $H_2S$ stimulated the production of one another and their accumulation is responsible to control the oxidative stress, for prevention of water loss via regulating the stomatal conductance, triggering the GSH accrual and stimulation of activities of SOD and CAT.

More recently, application of NO and $H_2S$ donors in *Solanum lycopersicum* plants has been reported to ameliorate the oxidative stress induced by high salinity[11]. This report suggested that $H_2S$ acted as a downstream signal for NO in this signaling pathway. In line, other reports have also reached to related conclusions relative to the mechanism of reaction to others stresses (such as heat) in which $H_2S$ acts downstream of NO signaling[23].

**NO and $H_2S$ in cadmium stress tolerance:** Being a harmful and non-essential metal, Cd is up taken by the plants roots and is collected in their several compartments which hampered their development and productivity[27]. The uptake/ accrual of Cd alters the absorption of essential nutrients and their allocation in the plant tissues[32]. It negatively affects the plants growth, biomass and water status, disturbs photosynthesis, decreases the photosynthetic pigments, hinders carbohydrate metabolism and indirectly triggers oxidative stress[42]. A large number of studies have shown that the exogenous application of some of the signal molecules such as NO and $H_2S$ can alleviate the toxic effects triggered by the exposure of heavy metals[16,20,21,32].

Plethora of literature clearly indicates that Cd stress-induces production of endogenous NO and $H_2S$, hence it is considered as important signaling substance in stress sensing and transmission and has been intensively explored for imparting roles in enhancing tolerance to Cd stress[21,42]. Shi et al[32] reported that Cd stress significantly hampered the germination and growth of plants and elicited the cell distortions in *Cynodon dactylon* L. On the other hand, NO and $H_2S$ significantly eliminated the Cd toxicity attributed by significant increase in biomass and lower electrolyte leakage and other oxidative markers. Also, these two signaling molecules activated the antioxidant defense mechanisms (SOD, CAT, POD, GSH and proline). NO might serve as an upstream signal for $H_2S$ production, hence pointing the control to antioxidant response for the amelioration of oxidative stress injury is caused by Cd[32].

**NO and $H_2S$ in arsenic stress tolerance:** In the recent decades, contamination of As in the irrigation water has become a global concern for the agricultural crop plants in many countries, where presence of As is more i.e. higher than that of permissible limit (10 μg $L^{-1}$) has been reported[2]. Toxicity of As could be eliminated to some extent using physiological processes. Hence, NO and $H_2S$ are renowned signaling molecules which are endogenously produced and can freely surpass the cellular membranes and have been intensively explored for their crucial roles in providing tolerance to plants against changing environments[16,33].

According to Singh et al[33] under As stress, exogenous application of $H_2S$ ameliorated the toxic effects of As on plant growth, biomass and photosynthesis with concomitant decrease in the content of As, ROS and oxidative injury and activated the members of AsA-GSH cycle. This may be attributed due to the following mechanisms:

1) Application of $H_2S$ increased NO accrual that directly scavenged ROS and the products of lipid peroxidation reaction and changed them into less harmful products and thus decreased the extent of oxidative stress.

2) NO and $H_2S$ served as signaling molecules and lessened the gathering of As which was evident in terms of better biomass accrual due to the stimulation in the AsA-GSH system and prevented oxidative injury by triggering antioxidant defense system.

3) NO and $H_2S$ both are signaling elements for activating antioxidant defensive mechanism that regulate the oxidative stress and

4) Addition of $H_2S$ activated the enzymes of the AsA-GSH pathway, which further maintained the redox balance of AsA and GSH in the cell and hence promoted the growth and biomass[33].

## Conclusion

Signaling triggered by NO and $H_2S$ is a general reaction in plants against abiotic stresses that is responsible for the acquirement of cross-adaptation. Moreover, when these signal molecules are applied exogenously, they elicit corresponding signaling, thereby imparting stress tolerance, thus NO and $H_2S$ are key signal molecules involved in cross-adaptation of the plants. Various studies demonstrate that treating the plants with these molecules perks up their defensive capacity by triggering their own antioxidative mechanisms. Moreover, the signaling cascade elicited by NO and $H_2S$ is not fully understood.

Available study showed that $H_2S$ could act either upstream or downstream of NO signaling depending on the plant processes such as closing of stomata, or in responses to abiotic stresses respectively. This review briefly focused the recent improvement on the relation of NO and $H_2S$ to impart abiotic stress tolerance in plants. However, further researches are needed to subtle the interacting roles of NO and $H_2S$ and other plant signals.

## Acknowledgement

We are very grateful to the Department of Science and Technology, New Delhi for providing DST INSPIRE fellowship to Vibhuti Chandrakar (No. DST/INSPIRE Fellowship/2013/791, dated 23.01.2013) and for DST-FIST scheme (No. 2384/IFD/2014-15, dated 31.07.2014).

## References

1. Alavi S.M.N., Arvin M.J. and Kalantari K.M., Salicylic acid and nitric oxide alleviate osmotic stress in wheat (*Triticumaestivum* L.) seedlings, *Journal of Plant Interactions*, **9,** 683-688 **(2014)**

2. Chandrakar V., Naithani S.C. and Keshavkant S., Arsenic-induced metabolic disturbances and their mitigation mechanisms in crop plants: A review, *Biologia*, **71**, 367-377 **(2016)**

3. Chandrakar V., Yadu B., Meena R.K., Dubey A. and Keshavkant S., Arsenic-induced genotoxic responses and their amelioration by diphenyleneiodonium, 24-epibrassinolide and proline in *Glycine max* L., *Plant Physiology and Biochemistry*, **112**,74-86 **(2017)**

4. Chandrakar V., Pandey N. and Keshavkant S., Plant responses to arsenic toxicity: morphology and physiology, In Hasanuzzaman M., Nahar K. and Fujita M., eds., Mechanisms of Arsenic Toxicity and Tolerance in Plants, Springer, Singapore, 27-48 **(2018a)**

5. Chandrakar V., Dubey A. and Keshavkant S., Modulation of arsenic-induced oxidative stress and protein metabolism by

diphenyleneiodonium, 24-epibrassinolide and proline in *Glycine max* L., *Acta Botanica Croatica,* **77,** 51-61 **(2018b)**

6. Chandrakar V. and Keshavkant S., Growth and metabolic responses of *Glycine max* L. to arsenate and arsenite: a comparative assessment, *Bangladesh Journal of Botany,* **47,** 105-113 **(2018a)**

7. Chandrakar V. and Keshavkant S., Nitric oxide and dimethylthiourea up-regulates pyrroline-5-carboxylate synthetase expression to improve arsenic tolerance in *Glycine max* L., *Environmental Progress and Sustainable Energy,* **38**, 402-409 **(2018b)**

8. Chen J., Wang W.H., Wu F.H., He E.M., Liu X., Shangguan Z.P. and Zheng H.L., Hydrogen sulfide enhances salt tolerance through nitric oxide mediated maintenance of ion homeostasis in barley seedling roots, *Science Reports,* **5**, 12516 **(2015)**

9. Corpas F.J., González-Gordo S., Cañas A. and Palma J.M., Nitric oxide and hydrogen sulfide in plants: Which comes first?, *Journal of Experimental Botany,* **70,** 4391-4404 **(2019)**

10. Da-Silva C.J. and Modolo L.V., Hydrogen sulfide: a new endogenous player in an old mechanism of plant tolerance to high salinity, *Acta Botanica Brassica,* **32,** 150-160 **(2018)**

11. Da-Silva C.J., Mollica D.C.F., Vicente M.H., Peres L.E.P. and Modolo L.V., NO, hydrogen sulfide does not come first during tomato response to high salinity, *Nitric oxide,* **76,** 164-173 **(2018)**

12. Fu P., Wang W., Hou L. and Liu X., Hydrogen sulfide is involved in the chilling stress response in *Vitisvinifera* L., *Acta Societatis Botanicorum Poloniae,* **82,** 295-302 **(2013)**

13. Gill S.S. and Tuteja N., Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, *Plant Physiology and Biochemistry,* **48**, 909-930 **(2010)**

14. Hasanuzzaman M., Ku H., Nahar K., Bhuyan M.H.M.B., Mahmud J.A., Baluska F. and Fujita M., Nitric oxide-induced salt stress tolerance in plants: ROS metabolism, signaling and molecular interactions, *Plant Biotechnology Report,* **12,** 77-92 **(2018)**

15. Hasanuzzaman M., Nahar K., Alam M.M. and Fujita M., Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticumaestivum*) seedlings by modulating the antioxidant defense and glyoxalase system, *Australian Journal of Crop Science,* **6,** 1314-1323 **(2012)**

16. He H., Li Y. and He L.F., Role of nitric oxide and hydrogen sulfide in plant aluminum tolerance, *Biometals,* **32**, 1-9 **(2019)**

17. Ismail G.S.M., Protective role of nitric oxide against arsenic-induced damages in germinating mung bean seeds, *Acta Physiologiae Plantarum,* **34**, 1303-1311 **(2012)**

18. Kaya C., Akram N.A. and Ashraf M., Influence of exogenously applied nitric oxide on strawberry (*Fragaria × ananassa*) plants grown under iron deficiency and/or saline stress, *Physiologia Plantarum,* **165**, 247-263 **(2019)**

19. Khan M.N., Mobin M., Abbas Z.K. and Siddiqui M.H., Nitric oxide induced synthesis of hydrogen sulfide alleviates osmotic stress in wheat seedlings through sustaining antioxidant enzymes, osmolyte accumulation and cysteine homeostasis, *Nitric Oxide,* **68,** 91-102 **(2017)**

20. Kharbech O., Houmani H., Chaoui A. and Corpas F.J., Alleviation of Cr(VI)-induced oxidative stress in maize (*Zea mays* L.) seedlings by NO and $H_2S$ donors through differential organ-dependent regulation of ROS and NADPH-recycling metabolisms, *Journal of Plant Physiology,* **219,** 71-80 **(2017)**

21. Li L., Wang Y. and Shen W., Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots, *Biometals,* **25,** 617-631 **(2012)**

22. Li Z.G., Min X. and Zhou Z.H., Hydrogen sulfide: a signal molecule in plant cross-adaptation, *Frontiers in Plant Sciences,* **7**, 1621 **(2016)**

23. Li Z.G., Yang S.Z., Long W.B., Yang G.X. and Shen Z.Z., Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings, *Plant Cell and Environment,* **36,** 1564-1572 **(2013)**

24. Ma D., Ding H., Wang C., Qin H., Han Q., Hou J., Lu H., Xie Y. and Guo T., Alleviation of drought stress by hydrogen sulfide is partially related to the abscisic acid signaling pathway in wheat, *PLoS One*, doi:10.1371/journal.pone.0163082, **11(9),** e0163082 **(2016)**

25. Mamaeva A.S., Fomenkov A.A., Nosov A.V., Moshkov I.E., Mur L.A.J., Hall M.A. and Novikova G.V., Regulatory role of nitric oxide in plants, *Russian Journal of Plant Physiology,* **62,** 427-440 **(2015)**

26. Min Y., Bao-Ping Q., Xue-li M., Ping W., Mei-ling L., Lu-lu C., Lei-tai C., Ai-qing S., Zhen-lin W. and Yan-ping Y., Foliar application of sodium hydrosulfide (NaHS), a hydrogen sulfide ($H_2S$) donor, can protect seedlings against heat stress in wheat (*Triticumaestivum* L.), *Journal of Integrative Agriculture,* **15**, 60345-60347 **(2016)**

27. Mostofa M.G., Rahman A., Ansary M.M.U., Watanabe A., Fujita M. and Tran L.S.P., Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice, *Science Reports,* **5,** 14078 **(2015)**

28. Pandey N., Chandrakar V. and Keshavkant S., Mitigating arsenic toxicity in plants: role of microbiota, In Hasanuzzaman M., Nahar K. and Fujita M., eds., Mechanisms of arsenic toxicity and tolerance in plants, Springer, Singapore, 27-48 **(2018)**

29. Peng R., Bian Z., Zhou L., Cheng W., Hai N., Yang C., Yang T., Wang X. and Wang C., Hydrogen sulfide enhances nitric oxide-induced tolerance of hypoxia in maize (*Zea mays* L.), *Plant Cell Reports,* **35,** 2325-2340 **(2016)**

30. Rughani G., Chandra J., Chandrakar V. and Keshavkant S., Lipid peroxidation and in-situ localization of ROS in assorted seeds exposed to salinity and artificial ageing, *Applied Science Reports,* **12,** 23-127 **(2015)**

31. Saxena I. and Shekhawat G.S., Nitric oxide (NO) in alleviation of heavy metal induced phytotoxicity and its role in protein nitration, *Nitric Oxide,* **32,** 13-20 **(2013)**

32. Shi H., Ye T. and Chan Z., Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response inbermudagrass (*Cynodondactylon* (L). Pers.), *Plant Physiology and Biochemistry*, **74,** 99-107 **(2014)**

33. Singh V.P., Singh S., Kumar J. and Prasad S.M., Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate-glutathione cycle: Possible involvement of nitric oxide, *Journal of Plant Physiology*, **181,** 20-29 **(2015)**

34. Wang Y., Li L., Cui W., Xu S., Shen W. and Wang R., Hydrogen sulfide enhances alfalfa (*Medicago sativa*) tolerance against salinity during seed germination by nitric oxide pathway, *Plant Soil*, **351,** 107-119 **(2012)**

35. Xalxo R., Yadu B., Chandra J., Chandrakar V. and Keshavkant S., Alteration in carbohydrate metabolism modulates thermotolerance of plant under heat stress, In Wani S.H. and Kumar V., eds., Heat stress tolerance in plants:physiological, molecular and genetic perspectives, First edition, 77-115 **(2020)**

36. Xalxo R., Yadu B., Chakraborty P., Chandrakar V. and Keshavkant S., Modulation of nickel toxicity by glycinebetaine and aspirin in *Pennisetumtyphoideum*, *Acta Biologia Szegediensis*, **61,** 163-171 **(2017)**

37. Yadu B., Chandrakar V. and Keshavkant S., Responses of plants towards fluoride: An overview of oxidative stress and defense mechanisms, *Fluoride*, **49,** 293-302 **(2016)**

38. Yadu S., Dewangan T.L., Chandrakar V. and Keshavkant S., Imperative roles of salicylic acid and nitric oxide in improving salinity tolerance in *Pisumsativum* L., *Physiology and Molecular Biology of Plants*, **23,** 43-58 **(2017)**

39. Yadu B., Chandrakar V., Korram J., Satnami M.L., Kumar M. and Keshavkant S., Silver nanoparticles modulated gene expressions, glyoxalase system and oxidative stress markers in fluoride stressed *Cajanuscajan* L., *Journal of Hazardous Materials*, **353,** 44-52 **(2018a)**

40. Yadu B., Chandrakar V., Meena R.K., Poddar A. and Keshavkant S., Spermidine and melatonin attenuate fluoride toxicity by regulating gene expression of antioxidants in *Cajanuscajan* L., *Journal of Plant Growth Regulation*, **37,** 1113-1126 **(2018b)**

41. Yadu B., Chandrakar V., Tamboli R. and Keshavkant S., Dimethylthiourea antagonizes oxidative responses by up regulating expressions of pyrroline-5-carboxylate synthetase and antioxidant genes under arsenic stress, *International Journal of Environmental Science and Technology*, **16,** 8401-8410 **(2019)**

42. Zhao H., Jin Q., Wang Y., Chu L., Li X. and Xu Y., Effects of nitric oxide on alleviating cadmium stress in *Typhaangustifolia*, *Plant Growth Regulation*, **78,** 243-251 **(2016)**.

(Received 16th March 2020, accepted 19th May 2020)