Nitric Oxide as a Savior Molecule Against Stress Induced by Chromium and Cadmium

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Abstract

Heavy metals (HMs) pollution is one of soil problems imposing great agronomic challenges leading to obstacles in ascertaining food safety. The deposition of HMs beyond permissible limits due to their uses in various agricultural, industrial and household purposes, greatly affect the soil fertility and physio-biochemical activities of plants growing in such soils. Among HMs, cadmium (Cd) and chromium (Cr) are very toxic, non-essential heavy metals whose concentrations have built-up recently in our environment due to several manmade activities. These elements enter into living organisms either by absorption through plants or are directly consumed by animals or microbes. They induce various morphological and physio-biochemical changes in plants ranging from seed germination, growth, nutrient uptake, photosynthesis to hormonal interactions. Excess reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation are two important markers of nitro-oxidative stress during heavy metal stress. Plants counteract by activating defence responses by inducing expression of antioxidant enzymes like (CAT, SOD, APX) and antioxidant compounds like Vitamin C and GSH. Nitric oxide (NO) is one of the active gaseous molecules generated during nitro-oxidative stress induced by HMs like Cd and Cr. NO is generated in the plants by diverse enzymatic and non-enzymatic systems. The specific and dedicated enzyme system for NO generation is not yet identified in the plants. NO helps to counteract the effects of HMs by activating various biochemical machineries such as induction of antioxidant defence systems, protein nitrosylation, programmed cell death. It is the molecule that has very promising roles and need to be explored much in relation to heavy metals.

Keywords: Antioxidants, Cadmium, Chromium, Heavy Metals, Nitric Oxide.

Introduction

Heavy metals (HMs) are defined as metals having density higher than 5 g cm⁻³ and possess high atomic weight and have density at least five grams greater than that of water (Weast, 1984; Tchounwou et al., 2012). It is often assumed that HMs and toxicity are interrelated and for this reason, metalloids such as arsenic and antimony are also considered in HM group and are able to induce toxic effects in plants. HMs are considered to be highly toxic and in recent years there has been an increasing public concern about their deleterious effects. There are 35 metals of concern and out of which 23 are HMs namely antimony (Sb), arsenic (As), bismuth (Bi), cadmium (Cd), cerium (Ce), chromium (Cr), cobalt (Co), copper (Cu), gallium (Ga), gold (Au), iron (Fe), lead (Fe), manganese (Mn), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), tellurium (Te), thallium (TI), tin (Sn), uranium (U), vanadium (V) and zinc (Zn) (Jaishankar et al., 2014).

HMs in the environment come from effluents of the industries, agriculture, domestic, geogenic weathering (He et al., 2005). At present, unplanned industrialization and anthropogenic activities have resulted in the contamination of HMs in the environment. Industrial effluents are loaded with heavy metals and these are discharged into the water bodies making them polluted and toxic (Akpor et al., 2014; Dietler et al., 2019). One of the most important sources of heavy metal pollution is the tannery industry and responsible for adding up of toxic HMs such as cadmium and chromium (Gowd and Govil, 2008; Whitehead et al., 2019) which get accumulated in vegetable tissues in high concentration (Nigussie et al., 2012). In addition to this, chemical fertilization in agricultural fields is also responsible of adding up of HMs (Gimeno-Garcia et al., 1996). The gradual buildup of HMs in the atmosphere lead to serious health problems as outbreak of a number of diseases (Liang et al., 2017). Apart from causing health problems to human beings, the HMs also subject the plants to lot of stress technically called as HMs stress. Early manifestations of HMs stresses are the oxidative stress (Shahid et al., 2014) as well as nitrosative stress (Georgiadou et al., 2018). They induce diverse physiological changes within the plant that ultimately affects the productivity (Rucinska-Sobkowiak et al., 2010; Dutta et al., 2018).

It is established that HMs induce oxidative and nitrosative stresses in plants and shows very intricate relationship between them. One of the key molecules involved in oxidative-nitrosative stress is the nitric oxide (NO). NO is an inorganic di-atomic gaseous signalling molecule. Its presence is now well reported ranging from microscopic organisms to higher plant systems.


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NO signalling and physiological role is now well established in animal system too (Donald et al., 2015; Yang et al., 2018; Astier et al., 2019). Plant NO emission was first observed in soybean plants treated with herbicides (Klepper, 1967; Mayer et al., 2018). After that, NO research gained momentum in plants when first report came with its role in plant defence (Durner et al., 1998; Bellin et al., 2013). Since then, NO has become an active area of research in plant biology. It acts as a signalling molecule and induces various physiological responses such as flowering (Zhang et al., 2019), senescence (Braund and Melihoç, 2019), root development (Sharma et al., 2019) posttranslational modification (S-nitrosylation) that regulates gene transcription (Mengel et al., 2013), gene expression, the mobilization of second messenger (Astier and Lindermayr, 2012), programmed cell death (He et al., 2019; Huang et al., 2019), seed germination (Pandey et al., 2019), stomatal movements (Neill et al., 2008; Fan and Liu, 2012), seed dormancy (Nagel et al., 2019). It is well reported in various abiotic stress responses such as drought, salinity, low and high temperature, UV and ozone exposure, heavy metals (HMs), mineral deficiency as well as pathogen and herbivory induced biotic stress. Cross talk of NO with various plant hormones has also been documented (Asgher et al., 2017; Zhu et al., 2019). In plants, NO is generated by both enzymatic and non-enzymatic systems (Corpas et al., 2009) and studies confirm that there is great modulation in pattern of NO generation and signalling during HMs stress condition particularly with Cr and Cd (Wei et al., 2020).

**NO Generating Enzymes in Plants**

NO generation is reported in various species ranging from unicellular bacteria to highly evolved angiosperms. NO is reported to be generated by both enzymatic and non-enzymatic systems in plants (Corpas et al., 2009) (Fig. 1). Despite this, no dedicated enzyme is reported in plant system till date. Mammalian nitric oxide synthase (NOS) enzyme (EC 1.14.13.39) was identified and described in 1989 for the first time. It has three isoforms in human (Pradhan et al., 1996). Further, by the use of immune-technique, radio labelling and NOS enzyme inhibitors, mammalian NOS enzyme-like activity has been reported in fungi like Flammulina velutipes (Wu et al., 2014), Phycycomycetes blankesleeanaus (Maier et al., 2001) and Neurospora (Filippovich et al., 2020) involved in biological roles such as apoptosis, secondary messenger, cellular development, morphogenesis, sporulation, spore germination and reproduction.

The presence of mammalian like NOS enzyme in plants has been detected by use of mouse anti-NOS antibody from the brain (nNOS) and western blot analysis in yeast and wheat germ (Kuo et al., 1995; Sen and Cheema, 1995), roots and nodules of Lupinus albus (Cuteo et al., 1996). Further, by the use of immune-technique, radio labelling and NOS enzyme inhibitors, mammalian NOS enzyme-like activity has been reported in maize (Riberro et al., 1999; Delledonne et al., 1998), tobacco (Nejamins et al., 2020), cotyledon of soybean (Modolo et al., 2002), dermal layer structure such as guard cell and epidermal cell of Kalanchoe daigreothiana, callus of gymnosperm Taxus brevifolia (Pedroso et al., 2000), Taxus cuspidata (Gong and Yuan, 2006) and Indian yellow mustard (Brassica juncea) that showed activation by protein kinase C (PKC) with Ca²⁺ (Talwar et al., 2012). Apart from the cytosol, mammalian like NOS enzyme has also been reported in cell organelles such as peroxisome as well as chloroplast (Barraso et al., 1999) epidermal cytosal, guard cell chloroplast and leaf parenchyma cell (Pedroso et al., 2000) nucleolar region of Nicotiana (Foisnser et al., 2000).

Recently, an extensive analysis was made for the presence of NOS homologs by analyzing transcriptome and genome of >1300 species of plants where it was found that 15 complete sequences show enough similarity with NOS. Interestingly, all

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**Fig. 1:** Mode of generation of NO in plants by enzymatic and non-enzymatic mechanisms.
belonged to algal species and no homologs from land plants raising questions on the presence or absence of NOS-like enzyme which requires more analysis (Jeandroz et al., 2016).

Nitrate Reductase (NR)
Nitrate as a source of nitrogen is absorbed by plant roots and assimilated by the reduction of nitrate to nitrite in the cytosol by the help of enzyme nitrate reductase (NR) (Tejada-Jiménez et al., 2019). NR is a homodimer, each with a molecular mass of 100 kDa in higher plants (Mohn et al., 2019). Each subunit contains three prosthetic groups, FAD (Flavin adenine dinucleotide), heme and a molybdenum complex (Lea, 1999). Molybdenum is bound to the enzyme via which acts pterin and act as a chelator of metal (Sparacino-Watkins et al., 2014).

\[ \text{NO}_3^- + \text{NADPH} + 2e^- \rightarrow \text{NO}_2^- + \text{NAD} (P)^+ + H_2O \]

The generated nitrite is acted upon by NR and its partner protein nitric oxide-forming nitrite reductase (NOF NR) to produce NO. NOF NR is an amidoxime reducing component (ARC) protein family (Havemeyer et al., 2006; Jeandroz et al., 2016).

NR was considered as an alternate enzyme to produce NO (Yamasaki et al., 1999) in spinach (Rockey et al., 2002), Chlamydomonas reinhardtii (Sakihama et al., 2006) and Arabidopsis (Wilkinson and Crawford, 1993). NR under hypoxia reduces nitrite into NO (Planchet et al., 2005; Gupta et al., 2012) and formation of NOx like product in vivo has been reported three decade ago in soybean (Harper, 1981) by using of NR mutants and comparing with wild-type soybean (Dean and Harper, 1986). The NR activity was absent in soybean NR mutant (Dean and Harper, 1986) nia1, nia2 mutants of Arabidopsis and cc-2929 strain of Chlamydomonas reinhardtii. NR also helps in NO generation by supplying nitrite to other enzymes located on cellular plasma membrane (PM-NiNOR) (Stöhr et al., 2001) or xanthine oxidase located in the peroxisome (Zhang et al., 1998).

Plasma Membrane Bound Ni-NOR (PM-NiNOR)
PM-NiNOR enzyme reported from the purified plasma membrane of tobacco (Nicotiana tabaccum L. cv. Samsun) is responsible for the generation of NO where reduction of NO\textsubscript{2} to NO occurs at pH 6.0. It requires reduced cytochrome C as an electron donor. Enzyme activity was not detected in soluble protein fraction or in plasma membrane vesicle of leaves and found insensitive to cyanide and anti-NR IgG and thereby indicating it to be different from PM-NR as showing molecular mass 310 kDa in comparison to 200 kDa of PM-NR (Stöhr et al., 2001; Jeandroz et al., 2016).

Xanthine Oxidoreductase (XOR)
NO generation occurs in plant peroxisome by xanthine oxidoreductase via reduction of nitrite into NO, during the anaerobic condition by using NADH or xanthine as reductant and has been reported in pea and white lupine (Gupta et al., 2011).

Polyamine Oxidase
The role of polyamine oxidase has been linked with the oxidation of polyamines like spermine or spermidine, seen dependent on availability of L-arginine, region-specific. Such NO generation showing a higher level in the elongation zone of the root tip and in primary leaves (veins and trichomes), while cotyledons had little or no effect (Tun et al., 2006). These results were based on NO generation detection by polyamine application and till date no such enzyme has been biochemically characterized (Gupta et al., 2011).

NO and Heavy Metal Stress
The presence of HMs in the soil causes toxicity that leads to decline in the crop plant growth and development and consequently productivity. NO molecules are generated during heavy metal stress and counteract either by removing HMs-induced ROS by direct scavenging or by stimulating antioxidants defence mechanism as seen in sunflower (Laspin et al., 2005; Groß et al., 2013). When an imbalance occurs between NO and ROS concentrations or/and antioxidant system due to strong HM stress, leads to oxidative stress and nitrosative or combination of both i.e., nitro-oxidative stress (Corpas and Barroso, 2013). Along with cytosolic enzymes, different spaces and organelles such as in apoplast, chloroplasts, mitochondria, endoplasmic reticulum and peroxisomes also show responses by generating NO parallel to ROS synthesis during HMs stress (Sahay and Gupta, 2017). Chromium (Cr) and Cadmium (Cd) are the two most non-essential, toxic HMs, rapidly taken up by plants and accumulated in various plant organs that inhibit the growth and development of plant (Huybrechts et al., 2019; Sharma et al., 2020; Genchi et al., 2020).

Chromium Contamination and Toxicity
Due to wide industrial use and unplanned disposal, chromium contamination has become a matter of great concern. Its deleterious effect is noticed both in plants and in animals. The toxic effect of chromium in plant growth includes changes in germination pattern, overall growth of plants, dry matter production and yield. All these changes are linked to alteration in physiological processes (Shanker et al., 2005). The deleterious physiological effects of chromium are greatly discussed by Sharma et al. (2020) and presented in selected crop plants is tabulated in Table 1.

Effect of Chromium on Nutrient Uptake
Chromium exposure affects nutrient uptake by various means such as forming insoluble compounds that in turn affects the absorption of many essential nutrients (Fe, Mg, P, Ca) or enhancement of concentration of certain nutrients (Mn and P) disturbing nutrient balance observed in Citrus and paddy plants (Oryza sativa L.) (Seneviratne et al., 2019), causes nutrient deficiencies or imbalance in rice (Zeng et al., 2010), phytotoxicity in Radish (Raphanus sativus) (Tiwari et al., 2013). Cr toxicity also affects decline in the root growth and impairment of the root penetration, gets accumulated in vegetable tissues at higher concentrations seen especially soil receiving leather industry effluents (Nigusie et al., 2012).

Effects of Chromium and Photosynthesis
Chromium shows multiple effects on photosynthetic machinery and causes decline in chlorophyll biosynthesis in Nymphaea alba L. (Vajpayee et al., 2000), total chlorophyll content, chlorophyll a (Chl a) and chlorophyll b (Chl b) pigment accumulation of Catharanthus roseus plants by inhibiting
Table 1: Deleterious effect of chromium in selected crop plants

<table>
<thead>
<tr>
<th>Name of the plants</th>
<th>Effects of chromium toxicity</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Rice</td>
<td>1. Reduction in plant height, elongation of the root and biomass accumulation. 2. Damage to root cells. 3. Alteration on chloroplast structure. Swollen stroma/grana lamellae, reduction in grana stacking per chloroplast. 4. Distorted meristematic cells, disappearance of nucleolus and enlarged vacuolar volume.</td>
<td>Qiu et al., 2013</td>
</tr>
<tr>
<td>Wheat</td>
<td>1. Reduction in leaf length, fresh mass, dry mass and germination. 2. Decline in the number of reaction centres and rate of electron transport of PS-II.</td>
<td>Mathur et al., 2016</td>
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<tr>
<td>Maize</td>
<td>1. Reduction in leaf area, cob (Central core of ear of corn) formation, 100-grain weight (total weight of hundred grains of the plants taken at random), shoot fresh biomass, and yield formation. 2. Increase in content of free proline, soluble sugars and total phenolic contents and decreased soluble protein content. 3. Enhanced lipid peroxidation and electrolyte leakage. 4. Hyperactivity of antioxidative enzymes.</td>
<td>Anjum et al., 2017</td>
</tr>
<tr>
<td>Barley</td>
<td>1. Reduction in plant growth, plant height, root dry weight, shoot dry weight, the number of tillers. 2. Deposition of chromium crystals along the cell walls, shrinkage of the cell membrane, disappearance of nucleolus, and disruption of the nucleus and nuclear membrane.</td>
<td>Ali et al., 2013</td>
</tr>
<tr>
<td>Sorghum</td>
<td>1. Reduced plant height, dry weight, fresh weight, shoot length, root length, grain yield and chlorophyll content. 2. Increase in activity of antioxidant enzyme. 3. Increase in content of glutathione, ascorbate and proline.</td>
<td>Kumar et al., 2019</td>
</tr>
<tr>
<td>Chick pea</td>
<td>1. Reduction in coleoptile and radical length along with size of the seeds. 2. Reduction in germination. 3. Reduction in root length. 4. Decrease in photosynthetic pigment and carotenoids. 5. Distorted xylem and phloem.</td>
<td>Medda and Mondal, 2017</td>
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<tr>
<td>Mustard</td>
<td>1. Significant decrease in chlorophyll and carotenoid content. 2. Reduction in stomatal density and distortion of morphology. 3. Increase in lipid and water-soluble antioxidants.</td>
<td>Handa et al., 2018</td>
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</tbody>
</table>

Chromium and Seed Germination
Chromium also reduces seed germination by causing deleterious effects on hydrolyzing enzymes such as amylase. Furthermore, Cr has inhibitory effects on acid phosphatases phytase and ribonuclease, with no significant effects on amylase and protease (Dua and Sawhney, 1991).

Chromium, NO, Nitro-Oxidative Stress and Antioxidant System
Maintaining balanced metabolic functions under stress conditions is essential and crucial for plants to survive. HMs stress induces the generation of ROS and RNS such as hydroxyl radicals (OH), hydroperoxyl radicals (HOO), superoxide (O2−), the peroxinitrite (ONOO−) ion, the paramagnetic singlet oxygen (O2•), NO, hydrogen peroxide (H2O2), ozone (O3) and hypochlorous acid (HOCl) molecules and consequently affects the diverse physiological process of plants seen such as lipid peroxidation, photosynthetic and respiratory damage and elevated level of malondialdehyde (MDA) and H2O2 (Hasanuzzaman et al., 2020). Therefore, a balance between generation and scavenging of ROS and RNS is required. It is achieved by regulating the production of enzymatic and non-enzymatic antioxidants. Hence, the ability of plants to cope with nitro-oxidative stress is characterized by the generation of high degree of antioxidants for the detoxification of harmful ROS and RNS (Mahmud et al., 2019). However, these antioxidants may vary with the duration, plant species, and tissues under stress conditions.

Cr interacts with the catalytic site or any other site of the enzyme, deactivates and thereby inhibits crucial enzymes involved for maintaining cellular homeostasis consequently ROS scavenging is hampered leading to cellular damages. On the other hand, Cr binds and utilizes the reduced form of glutathione (GSH) and its derivatives that help in ROS amelioration affecting cellular functions.

Plasma membrane-bound NADPH oxidase also shows a positive contribution in oxidative stress in response to Cr stress causing damage to DNA, lipids, pigments, proteins that affects functioning of the plasma membrane. Cr stress influences various anti-oxidative enzymes (SOD, CAT, GPX, MDHAR, DHAR, GR and GSH) that in turn quench ROS (Hasanuzzaman et al., 2020). Superoxide dismutase (SOD) helps in dismutation of...
superoxide radical; catalase (CAT) is involved in the dismutation of $\text{H}_2\text{O}_2$ to $\text{O}_2$ and $\text{H}_2\text{O}$ and guaiacol peroxidase (GPX) is the key enzyme involved in ameliorating the damaging effect of cellular ROS.

NO is seen as the most important RNS in plants. NO accumulation leads to activation of the antioxidant defence system, reduction in ROS, and ultimately redox balance. It is found that $\text{H}_2\text{O}_2$ is vital for ABA-mediated NO production via the NR activity (Qiao et al., 2014) and plays a crucial role in signal transduction as well as phytotoxicity. NO generated in Cr stressed tomato plants protected tomato roots by sequestering Cr into vacuoles and up-regulated ascorbate-glutathione cycle and glutathione biosynthesis (Kushwaha et al., 2020). NO reacts with $\text{O}_2^-$ and forms peroxynitrite (ONOO$^-$) that causes post-translational modification (PTM) in tyrosine residue of proteins or nitrosative alteration that leads to dysfunction of peroxisomal NADH-dependent hydroxy pyruvate reductase, inhibition of enzymes (Glycylate oxidase, CAT) or regulates $\text{H}_2\text{O}_2$ level. Such cascading actions show the complex interaction of NO generated under metal stress (Hasanuzzaman et al., 2020). Along with these antioxidants, plants facing HMs stress also synthesize and secrete low molecular weight non-enzymatic antioxidants for reducing oxidative stress, such as ascorbic acid, cysteine, glutathione, non-protein thiol and proline, which works by scavenging ROS (Rizvi et al., 2020) (Fig. 2).

**Cadmium Toxicity in Plants**

Cadmium generally occurs at a concentration of 0.2 mg kg$^{-1}$ in the lithosphere, 0.3 mg kg$^{-1}$ in the sedimentary rocks and 0.53 mg kg$^{-1}$ in the soil. It is a non-essential element and is highly toxic to both animals and plants (Liu et al., 2013). Cadmium contamination in the soil and consequent transfer in food crops is a serious environmental problem and is a consequence of unplanned urbanization, uncontrolled and intensive agricultural practices. It poses high threat to soil quality, food safety, human and animal health (Khan et al., 2017). Cadmium affects plants negatively and can alter the uptake of minerals by affecting the availability of minerals in the soil. It also affects the mineral availability by reducing the microbial population (Shentu et al., 2008; Shahid et al., 2017). Cr induces nitro-oxidative stress in plants (Hasanuzzaman et al., 2020) and affects physiological processes such as plant hormone regulation, stomatal behaviors, transpiration, photosynthesis and chlorophyll content are also affected by cadmium (Genchi et al., 2020).

**Cadmium and Hormones**

Cd exposure to plants causes disturbance in plant hormones levels. Auxin level was found low when plants were exposed with Cd and associated with reduced PIN1/3/7 protein without reduction in its transcripts. Cd also stabilized AXR3/IAA17 protein that represses auxin signalling in the Cd-mediated process. NO scavenger and inhibitor-based decrease in Cd-induced NO accumulation affected the Cd-mediated inhibition of root meristem development, reduction in auxin and PIN1/3/7 accumulation as well as stabilization of AXR3/IAA17 indicating that NO participates in Cd-mediated inhibition of root meristem growth (Yuan and Huang, 2016). However, Cd treatment to tolerant *Oryza sativa* L. seedlings showed an increase in ABA level (Hsu and Kao, 2003). On the other hand, NO donor (SNP) showed the dichotomous results as up-regulating the levels of jasmonic acid (JA) and proline content in plant tissues, down-regulating the ethylene level in shoots and roots both and the levels of salicylic acid in roots only. It also improved mineral absorption, regulation of proton pumps, and maintained hormone equilibrium by eliminating oxidative stress in conditions where Cd-induced toxicity led to NO depletion (Liu et al., 2015a). Gibberellic acids (GAs)-alleviated Cd toxicity through the reduction of the Cd-dependent NO accumulation and expression of Cd$^{2+}$ uptake related gene-IRT1 in *Arabidopsis* (Zhu et al., 2012).

**Cadmium and Biomass**

High concentration of Cd (100μM) markedly reduced biomass, NO production and chlorophyll (Chl a, Chl b and total Chl) concentration and stimulated ROS that was overcome by the treatment of NO donor SNP (50μM). SNP (50μM) led to biomass accumulation, reduced level of $\text{H}_2\text{O}_2$ and MDA, stimulated ROS-scavenging enzymes and mitigated the $\text{H}^+$-ATPase inhibition in proton pumps in *Trifolium repens* (Liu et al., 2015b). S-nitrosylation is involved in the concentration-dependent ameliorating effect of SNP against Cd toxicity in leaves of *Boehmeria nivea* (L.) Gaud. (Wang et al., 2015). Cd contamination affected germination, growth, yield, quality and nutrients in rice plants and induced plants to increase the production of glutathione (GSH), asbsciscic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and nitric oxide (NO) (Chen et al., 2019).

**Cadmium and Photosynthesis**

Cd hampered photosynthesis and the growth of various plant species. NO is reported to be involved in the plant response to Cd stress (Gill et al., 2013; Hasanuzzaman et al., 2020) and counteracts Cd-induced cytotoxic processes mediated by ROS in *Brassica juncea* through cross-talk between ROS, NO and antioxidant responses (Verma et al., 2013) and modulates protein changes in the plasma membrane. Exogenous NO could promote the scavenging of ROS, keep the mineral nutrition in balance, alleviate the damage to the photosynthetic apparatus, making the tomato seedlings with their optimum
Decrease in plant height, leaf number, root number and volume, and biomass.
2. Reduction in shoot and root length.

Oryza sativa, 1992), evolution alongside a
1. Chlorosis and decrease in chlorophyll content.
2. Increase in levels of hydrogen peroxide and MDA.
3. Decrease in levels of CAT.
4. Increase in activities of SOD and POD.

S, improved plant ability
2. Leakage of electrolyte.
3. Increase in antioxidant enzyme activity namely SOD, POD, CAT and GPx.
4. Decrease in grain yield.

Chloroplast degradation and increase in chlorophyll content.
2. Leakage of electrolyte and increase in hydrogen peroxide and MDA content.
2. Overall reduction in biomass production.

Maize
1. Oxidative stress as evident from an increase in MDA and hydrogen peroxide levels.
2. Increase in levels of CAT.
3. Decrease in protein content and increase in proline content.
4. Increased MDA levels.

Barley
1. Reduction in plant height, leaf number, root number and volume, and biomass.
2. Leakage of electrolyte and increase in hydrogen peroxide and MDA content.
2. Reduction in dry biomass.
3. Overproduction of reactive oxygen species and signs of lipid peroxidation.
4. Significant decrease in activities of antioxidant enzymes.

Table 2: Deleterious effect of cadmium in selected crop plants.

<table>
<thead>
<tr>
<th>Name of the plants</th>
<th>Toxic effects of cadmium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>1. Decrease in the length of roots, shoots, seedling and dry weight of roots, shoots and seedlings. 2. Increase in levels of hydrogen peroxide and MDA. 3. Decrease in levels of CAT. 4. Increase in activities of SOD and POD.</td>
<td>Çatav et al., 2020</td>
</tr>
<tr>
<td>Maize</td>
<td>1. Oxidative stress as evident from an increase in MDA and hydrogen peroxide levels. 2. Leakage of electrolyte. 3. Increase in antioxidant enzyme activity namely SOD, POD, CAT and GPx. 4. Decrease in grain yield.</td>
<td>Anjum et al., 2015</td>
</tr>
<tr>
<td>Sorghum</td>
<td>1. Reduction in stem length and leaf number. 2. Leakage of electrolyte and increase in hydrogen peroxide and MDA content.</td>
<td>Jawad Hassan et al., 2020</td>
</tr>
<tr>
<td>Chick pea</td>
<td>1. Reduction in root length, shoot length and plant height. 2. Overall reduction in biomass production.</td>
<td>Ullah et al., 2020</td>
</tr>
<tr>
<td>Mustard</td>
<td>1. Reduction in plant height, shoot and root length. 2. Reduction in pigment concentration. 3. Decrease in protein content and increase in proline content. 4. Increased MDA levels.</td>
<td>Ahmad et al., 2015</td>
</tr>
</tbody>
</table>

HMs are an enemy for photosynthesis (Prasad and Strzalka, 1999) as they disrupt enzyme activities, oxidize PS II, disorganize the electron transport chain and mineral metabolism. Cd caused reduction in chlorophyll and heme levels of germinating seedlings of Phaseolus vulgaris (Somasekharaiyah et al., 1992), increase in lipid peroxide levels, a dose-dependent induction of lipoxygenase activity, growth reductions, inhibition of chlorophyll content and photosynthesis in wheat (Ouzounidou et al., 1997). Cd inhibited the leaf O$_2$ evolution alongside a reduction in photosynthesis (Gill and Tuteja, 2011). SNP exerted an advantageous effect by alleviating the inhibitory effects of Cd on seed germination and seedling growth which might have interacted with NO in rice (Oryza sativa L.) (He et al., 2014). Deleterious effects of cadmium have been listed in Table 2.

Effect of Cadmium in Seed Germination and Seedling Growth

Cd inhibits germination of various plant species by inhibition of physiological and metabolic activities as reported in the seeds of Sorghum bicolor (Poaceae), rice seeds (Barceló and Poschenrieder, 1990), due to inhibition of root coleoptile growth, inhibition of carbohydrate hydrolysis and the translocation of hydrolyzed sugars in seedlings (Kuriakose and Prasad, 2008). A significant decrease in rice seed germination index, vigor index, root and shoot lengths as well as fresh weight as compared to control was observed in plants exposed to Cd (He et al., 2014) and exogenous NO donor SNP can balance the inhibitory effects in rice seed germination and seedling growth (Seneviratne et al., 2019). Various concentration of Cd treatment showed fluctuating antioxidant levels in rice seedling (Ali et al., 2002). When Cd and Cu present together, amylase, acid phosphatase and alkaline phosphatase enzyme activities declined in the endosperm of barley seeds (Kalai et al., 2014). As pea (Pisum sativum; Fabaceae) seeds were treated with a series of Cd concentrations, both α and β amylase activities were suppressed (Chugh and Sawhney, 1996). The starch degradation can be impeded even at low Cd levels due to the reduction in α-amylase activity. Changes in proteolytic enzymes in response to Cd stress have been studied in rice seeds. Increased Cd concentration stressed germinating rice seeds and elevated protein and amino acid level alongwith a reduction in protease activity. It also exhibited enhanced uptake of Cd in embryo axes than in the endosperm (Shah and Dubey, 1995). The accumulation of proline in seedlings in response to Cd was reported in Cajanus cajan, Vigna mungo and Triticum aestivum (Saradhi, 1991). It supplies extra energy via mitochondria to overcome stress (Charest and Ton Phan, 1990).

Cd, NO, Nitro-Oxidative Stress and Antioxidant System

NO involved in response to Cd modulates protein expression in plants. Use of quantitative proteomics approach based on isobaric tags for relative and absolute quantification (iTraq) to identify differentially regulated proteins in rice plasma membrane showed modulations in proteins involved as ATPases, kinases, metabolic enzymes, phosphatases, phospholipases (PLD), and transporters. NO donor (SNP) caused enhancement in Cd-induced PLD activity and accumulation of phosphatidic acid (PA), elevated antioxidant enzymes activities and enhanced glutathione accumulation (Yang et al., 2016).
Exogenous application of NO donors ameliorated Cd-induced toxicity by minimizing oxidative stress, re-establishing ATPase activity and maintaining stress-related hormones in white clover plants (Liu et al., 2015a). High concentrations were of Cd caused significant decrease in total phenolic, GSH and NO levels when compared with control in maize plants (Akinyami et al., 2017). GSH regulates Cd stress tolerance. It was confirmed by GSH biosynthesis inhibitor buthionine sulfoximine (BSO) that aggravated stress. The stress develops by enhancing ROS level, lowering NO and S-nitrosothiol content in tomato plants (Hasan et al., 2016). GSH works by dual approaches, viz., chelation and sequestration of Cd and stimulating NO, SNO and antioxidant machinery. It has also been observed that Arabidopsis HY1 confers Cd tolerance by decreasing NO production and improving iron homeostasis (Han et al., 2014). Alleviating role of H$_2$S and NO is reported in Cd-induced oxidative damage in alfalfa seedling roots (Li et al., 2012) and Bermuda grass (Cynodon dactylon L. Pers) (Shi et al., 2014).

NO promotes Cd$^{2+}$-induced Arabidopsis PCD by promoting MPK6-mediated caspase-3-like activation in roots (Ye et al., 2012, 2013). In yellow lupine plants, signalling response was accompanied by the NADPH-oxidase-dependent superoxide anion production. On the other hand, Ca may alleviate Cd toxicity via the production of endogenous NO with variation in the levels of non-protein thiols, protein thiols and matrix polysaccharides (Zhang et al., 2012). However, NO improved the NR activity significantly and helped tomato seedlings to recover their biomass under Cu and Cu+Cd treatment. Both metals (Cu and Cd) induced lipid peroxidation via decreasing antioxidant enzymes. It suggests a different response and regulation mechanism that involves exogenous NO in tomato seedlings under Cu and Cd stress (Wang et al., 2016).

**Conclusion and Future Perspective**

HMs showing higher toxicity upon exposure to plants and other living organisms, have become a public concern due to their deleterious effects. Chromium and cadmium are non-essential toxic HMs which interfere in all the stages of plant growth and development. Cr and Cd show similar response in plants ranging from morphological, physio-biochemical responses but in differential rates and degrees. The effects have been seen on vegetative growths (root and leaf growth, cell wall formation, nutrient uptake) and reproductive growths (pollen tube germination and morphology, seed germination), epigenetic stage where DNA level alteration showing effects in next generation. These HMs are responsible for activating two phenomena’s namely induction of nitroso-oxidative stress and antioxidant systems depending upon time and tolerance capacity of plant species. NO is a recently identified nitrogen-based active gaseous molecule involved in various plant physiological responses induced during stress conditions including Cr and Cd stress. It works by improving the ROS and RNS scavenging capacity as well as inducing antioxidant system and phytochelatory mechanisms at cellular and organelle level. These actions of NO are significantly useful to minimize and manage the impact of metal toxicity in crop plants growing in contaminated soil and heavy metal loaded water used for irrigation. Another opportunity exists for plant science researchers as till date no dedicated NO generating enzyme has been reported in higher plant system. Discovery and dissection of such system will throw more in-depth understanding on NO biology in relation to HMs.

**References**


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NO as Savior Molecule against Cr and Cd Induced Stress


